# STELLER SEA LION (*Eumetopias jubatus)* STRANDINGS AND THE ROLE OF PATHOGENS IN REPRODUCTIVE FAILURE

By

Janessa A. Esquible, B.S.

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APPROVED:

Shannon Atkinson, Committee Chair

Kathy Burek-Huntington, Committee Member

Keith Cox, Committee Member

Sherry Tamone, Committee Member

Milo Adkison, Chair

*Graduate Program in Fisheries*

S. Bradley Moran, Dean

*School of Fisheries and Ocean Sciences*

Michael Castellini,

*Dean of the Graduate School*

Abstract

Steller sea lions (SSL, *Eumetopias jubatus)* have faced severe population fluctuations over the last five decades with a myriad of possibilities affecting their SSL population including disease, malnutrition, predation, climate change, entanglement in marine debris, and other factors. This thesis examined the effects that anthropogenic factors and disease may play in SSL strandings and reproductive failure. The goal of this study was to characterize long-term seasonality and spatial trends in SSL strandings and to investigate the role *Brucella* spp., *Coxiella burnetti. Chlamydophila* spp. and morbilliviruses may play in reproductive failure including spontaneous abortion and premature parturition. In Chapter 1, we utilized stranding data (n=1507) collected in Alaska, Oregon, and Washington from 1990-2015. We assessed temporal trends by identifying seasonality patterns across all years, analyzing sex, age class, body length, and characterizing signs of human interaction including factors contributing to mortality. Clear seasonality trends were evident, with the greatest number of reported strandings occurring during the spring and summer. Gunshot wounds and fishery interactions accounted for a large proportion (46%) of human interaction cases in strandings. Adult males were the most frequently stranded sex and age class in the Alaska and West Coast Regions. This study attempted to quantify efforts to monitor strandings and determined that the apparent increase in strandings following 2000 was likely due to increased stranding response effort resulting from increased federal grant awards. We encourage conducting further spatial analyses of strandings in addition to continued stranding surveillance monitoring with attempts to improve stranding response time.

In Chapter 2 of my thesis, we analyzed archived lung, skin lesion and placenta tissues for the pathogens of interest in SSL fetuses (n=18) and neonatal pups (n=2) collected from 1998-

2015 in Alaska. Associated pathological findings and morphometric data were examined to identify signs of pathology or abnormalities in all cases. Marine mammal *Brucella* was detected in the lung tissue of three cases. This is the first documented detection of *Brucella* in SSL by PCR methods. Phocine distemper virus was also detected in the skin lesion of two cases and in the placenta of one case, in which the cases with skin lesions exhibited abnormal pathology that included vesiculoulcerative dermatitis. Currently, there is very little available information on the significance of *Brucella* spp. and morbilliviruses in marine mammal populations inhabiting Alaskan waters. Therefore, this study demonstrates the clear need to continue disease surveillance programs and further investigate the role disease may play in SSL reproductive health, and more generally on cohort population stability.

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General Introduction

Steller sea lions (SSL; *Eumetopias jubatus)* are the largest members of the Otariidae family and are considered polygynous and long-lived (15-20 years; Fiscus 1961). SSL inhabit areas along the North Pacific rim from California to Japan with about 70% of the population dwelling in Alaskan waters (National Research Council 2003). Thus far, researchers have documented three different populations including the eastern, western and Asian SSL populations (Bickham et al. 1996; Loughlin 1997; Baker et al. 2005). The present study focuses on the eastern and western SSL populations, also referred to as distinct population segments (DPS) due to genetic and population trend differences, where the line of demarcation of the two stocks is at 144°W longitude (Bickham et al. 1996; Loughlin 1997).

SSL are semi-migratory, dispersing between breeding and non-breeding seasons, and congregated at rookeries during the breeding season (Jemison et al. 2013). Male SSL reach sexual maturity between the ages of five and seven, establishing breeding territories after the age of nine to thirteen (Gentry 1970; Sandegren 1970; Gisiner 1985; Smith 1988). In female SSL, first ovulation occurs on average at 4.6 years of age and females give birth between mid-May to mid-July (Pitcher et al. 1998). SSL exhibit embryonic diapause in which implantation of the embryo is delayed for up to 3.5 months, an evolved strategy that may allow for maximal offspring survival (Atkinson 1997, Pitcher and Calkins 1981). Furthermore, SSL may also abort a fetus to optimize reproductive success, especially in times of nutritional stress or environmental change (Pitcher et al. 1998).

The western and eastern DPS have shown notable differences in population abundance over the past several decades (Pendleton et al. 2006). Many studies have been conducted on the well-known decline that began in the 1960s, resulting in the listing of the western SSL stock as

endangered, and the eastern SSL stock as threatened under the US Endangered Species Act (Loughlin and York 2000; Miller et al. 2005; Atkinson et al. 2008). The decline is suspected to have started in the eastern Aleutian Islands and continued throughout the remainder of the Aleutian Islands and further expanded to the Gulf of Alaska in the late 1970s (Braham et al. 1980; Trites and Larkin 1996). Contrary to this, SSL populations were increasing in Oregon, British Columbia and Southeast Alaska during the same time period (Merrick et al. 1987; Trites and Larkin 1996; Calkins et al. 1999). There are numerous proposed hypotheses to the decline including disease, malnutrition, predation, climate change, entanglement in marine debris and other unknown causes suggesting it is multifactorial (Loughlin 1998; Trites and Donnelly 2003; Burek et al. 2005; Hennen 2006; Atkinson et al. 2008). The dichotomous trajectories of the eastern and western SSL stocks have encouraged the need for further analyses of factors that may have contributed to the SSL population decline and are playing a role in recently observed SSL population trends. The present study investigated the role anthropogenic factors and disease may have played in SSL strandings, spontaneous abortion and premature parturition. More specifically, the study was split into two chapters. One chapter focused on SSL stranding trends and the second chapter focused on the potential role *Brucella* spp., C. *burnetii, Chlamydophila* and morbilliviruses may have played in SSL spontaneous abortion and premature parturition.

A marine mammal stranding refers to an individual or group of animals that are found on a beach or shore and are unable to return to its natural habitat or observed dead on the shore and/or beach (Geraci and Lounsbury 2005). Depending on the quality of the data, information on marine mammal strandings can be of utility when investigating human interactions, environmental contaminants and natural disease (Gerber et al. 1993; Dierauf 1994). Pinniped stranding rates have also been correlated to variation in local abundance (Osinga et al. 2012).

The present study utilized SSL stranding data to better understand divergent population trajectories in the eastern and western DPS in addition to other anthropogenic and disease related factors that may have contributed to SSL strandings which occurred from 1990-2015 in Alaska, Oregon and Washington. SSL stranding data were obtained from two U.S. regional stranding networks: Northwest and Alaska Regional Stranding Networks. The National Marine Mammal Stranding Network provides a framework that requires the reporting of stranded animals, which facilitates the identification of mass mortalities or strandings caused by disease, toxins or other problems (NOAA 2016). For the purpose of the first chapter of my thesis, Level A reports were utilized. Level A data provide "basic minimum data", and corresponds to the information required on Level A stranding forms," ...which includes details of the stranding incidents such as animal ID, location of stranding, condition at examination, demographic information such as age class and sex, standard length, information on the purpose of samples collected and other occurrence details" (NOAA 2017). An additional human interaction form is encouraged to be completed, but only required for "code 1-3 cetaceans, endangered/threatened species, and large whales (NOAA 2017)," which, for the purpose of this study, would only be required for the western SSL DPS strandings. Volunteer stranding networks aid in the collection and documentation of data found in Level A reports (NOAA 2016). Cause of death or stranding cannot always be implied from this information alone; however, the type of human-interaction can be helpful in determining factors contributing to SSL strandings or mortalities.

The second chapter of the present study focuses on the role infectious disease agents may have played in SSL spontaneous abortion, premature parturition and neonatal deaths from 1998-2015 in Alaska. Prior studies suggested reproductive failure may have contributed to the SSL decline (Calkins and Goodwin 1988). Reproductive failure defined by Calkins and Goodwin

(1988) refers to embryo resorption, abortion or missed pregnancy, which occurs if the female ovulated and fertilization or implantation failed. Disease, stress and toxins, poor nutrition and genetic defects are possible factors to consider when identifying causes of reproductive failure (Burek et al. 2003). More specifically, disease may increase mortality by causing abortion, premature parturition, neonatal mortality, decreased fecundity and/or conception rates, which can have population level implications (Scott 1988; Gulland 1995). There could be a direct linkage between disease and reproductive failure that has been observed in other species, but remains largely unknown in SSL. Data utilized in the second chapter of my thesis elucidated the effects disease had on reproductive failure in aborted and premature SSL fetuses and neonates.

Archived lung, placentae, and skin lesion tissue samples were obtained from SSL aborted fetal cases, an intrauterine fetus and neonatal pups for the purpose of detecting *Brucella* spp., C. *burnetii, Chlamydophila* and morbilliviruses in these tissue samples. Additional data were obtained from necropsy reports conducted on these cases (n=20) which were used to determine abnormal findings suggestive of infection or other factors that may have played a role in reproductive failure. Unlike the Level A data utilized in Chapter 1, supplemental Level B and C data was used for the purpose of identifying signs of pathology, cause of death and other comprehensive data produced from necropsy reports. Level B data include "weather and tide conditions, offshore human/predator/prey activity, morphometrics, pre-stranding/stranding/rehabilitation behavior, stranding/rehabilitation health assessments, and life history samples" which are opportunistically collected at the time of a necropsy (NOAA 2017). Detailed data and results from the tissue collection at the time of the necropsy is referred to as Level C data (NOAA 2017).

Both chapters address disease and anthropogenic factors as potential contributors to the differences in population trends observed between the western and eastern SSL DPS through the use of stranding data and archived tissue samples. This study has broader implications when considering how stranding data may help with elucidating trends and issues occurring in nearshore environments, as the bulk of these strandings occurred in relatively close proximity to shore (Flint et al. 2015). Current unknown SSL mortality trends and presence of zoonotic infectious disease agents in commercially and ecologically vital locations can be identified (Bossart 2011). The present study covers a large geographic range and time period and performs analyses on SSL stranding data sets and fetal and neonatal archived tissue samples to investigate SSL stranding trends and infectious disease agents as a factor of reproductive failure.

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Chapter 1: Stranding trends of Steller sea lions (*Eumetopias jubatus)*[1]

**Abstract**

Steller sea lions (SSL, *Eumetopias jubatus)* have faced severe population fluctuations over the last five decades, rendering the need for retrospective study. By identifying long-term stranding trends of SSL we can develop a better understanding of factors contributing to mortality that may affect SSL population dynamics. We characterized spatial and temporal trends of SSL strandings (n=1507) in Alaska, Oregon, and Washington over a 25-year period. Stranding reports were obtained from the Alaska and Northwest Region's Marine Mammal Stranding Networks. Temporal trends were assessed by identifying seasonality patterns across all years (1990-2015), analyzing sex, age class, standard body length, and characterizing signs of human interaction including factors contributing to mortality.

An apparent increase in strandings occurred after 2000, but was likely an artifact of increased stranding response effort resulting from increased federal grant awards. Adult males were the most frequently stranded sex and age class in the Alaska and Northwest Regions. Clear seasonality trends were evident, with the greatest occurrence of reported strandings during the spring and summer. Gunshot wounds and fishery interactions accounted for a large proportion (46%) of human interaction cases. In Alaska, the southeast region had the highest concentration of stranding occurrences. In the Northwest Region, Oregon had the highest documented stranding occurrences. Despite caveats associated with stranding data, our findings suggest rapid and continued stranding response is imperative for a better understanding of cause-specific mortality trends and other factors contributing to stranding events.

[1] Esquible, JA and Atkinson SA. Submitted to Endangered Species Research Journal. In Review.

## Introduction

Steller sea lions (SSL; *Eumetopias jubatus)* inhabit areas along the North Pacific rim from California to Japan, with about 70% of the population dwelling in Alaskan waters (National Research Council 2003). Many studies have been conducted on the well-known decline that began in the 1960s, which resulted in the listing of the western SSL stock as endangered and the eastern SSL stock as threatened under the US Endangered Species Act (Loughlin and York 2000, National Research Council 2003, Atkinson et al. 2008). Despite numerous proposed hypotheses, researchers have yet to determine a sole cause leading to the decline (National Research Council 2003, Hennen 2006, Atkinson et al. 2008). Disease, malnutrition, predation, climate change, entanglement in marine debris, and other factors may have contributed to the decline; however, there are limitations on assessing the myriad of possibilities affecting SSL populations (Loughlin 1998, Trites and Donnelly 2003, Burek et al. 2005, Atkinson et al. 2008). The divergent trajectories of the eastern and western SSL stocks, with a lack of a robust recovery of the western SSL stock versus the recently recovered and delisted eastern SSL stock, demonstrate the need for further analyses of multiple factors driving SSL population dynamics over large spatial and temporal scales.

The present study utilizes SSL stranding data collected from 1990-2015 to better understand trends in the spatial and temporal distribution of stranding occurrences across coastal Alaska, Washington and Oregon. A marine mammal stranding refers to an individual or group of animals that are found on a beach or shore either unable to return to its natural habitat or observed dead (Geraci and Lounsbury 2005). Stranding data may be utilized to complement live surveys by providing seasonal information on population distribution (Maldini et al. 2005) and potential issues occurring in nearshore environments, as the bulk of these strandings occur close

to shore (Flint et al. 2015). Therefore, the present study contributes to our understanding of unknown SSL mortality trends and may give rise to conservation concerns associated with anthropogenic interactions or specific locations (Bossart 2011).

There are several entities involved in coordinating and maintaining stranding surveillance programs. Marine Mammal Health and Stranding Response Program (MMHSRP) and the National Marine Fisheries Service (NMFS) both play important roles in oversight and facilitation of the stranding surveillance program, often referred to as the National Marine Mammal Stranding Network. MMHSRP provides support for the coordination of stranding surveillance programs whereas NMFS provides oversight of marine mammal stranding activities facilitated by regional stranding coordinators (NOAA 2016). The National Marine Mammal Stranding Network appoints stranding coordinators in five regions within the United States to assist with coordination of reporting stranded animals and identification of mass mortalities or strandings caused by disease, toxins or other problems (NOAA 2016). NMFS funds eligible members of the National Marine Mammal Stranding Network through the form of the John H. marine mammal rescue assistance Prescott grant and other programs to conduct stranding responses and associated stranding activities (NOAA 2018). These members are referred to as stranding agreement holders or participants and permitted to respond to marine mammal strandings.

Reporting of marine animal strandings requires all members of stranding networks to collect Level A data, which include animal ID, location of stranding, condition at examination, demographic information such as age class and sex, standard length, information on the purpose of samples, collected reasons for the stranding response, and details associated with the stranding event (NOAA 2017). National Marine Mammal stranding networks aid in the collection and documentation of data found in Level A reports (NOAA 2016). The present study does not

utilize additional Level B and C data due to limited access and lack of standardization of these forms, resulting in inconsistent data collected across regions. Despite study constraints due to limited detail of pathological and histological data, Level A reports do provide information needed to detect basic information on life history, biology and general health of a population (NOAA 2017). "An additional human interaction form is encouraged to be completed, but only required for marine mammal species listed as endangered/threatened species and large toothed whales, baleen whales and all cetaceans that strand as alive, freshly dead or in a state of moderate decomposition (NOAA 2017)". Cause of stranding or death cannot be determined from this information alone.

The present study aims to reveal the occurrence and overall distribution of SSL stranding incidents over a broad geographic range by compiling and synthesizing SSL stranding data collected between 1990 and 2015 from Alaska, Washington, and Oregon. These data were obtained from the Alaska Region and the Northwest Region National Marine Mammal Stranding Networks and the Alaska Department of Fish and Game. The long time period encompassed in these reports enable a thorough characterization of trends in SSL strandings, as typified in other stranding studies (MacLeod et al. 2004; Maldini et al. 2005). The main objectives of this study were:

*1)* To examine spatial trends in SSL strandings to aid in identifying geographic areas that may need improved stranding surveillance systems due to their lack of representation in the stranding reports.

*2)* To detect geographic areas of high stranding occurrences.

*3)* To identify annual and seasonal trends across a 25-year time period for both stocks.

4) To identify signs of human interaction for strandings across the Northwest and Alaska regions, some of which may be categorized as contributing factors to SSL stranding mortalities.

These objectives may aid in refining our current understanding of potential impediments to the recovery of the western SSL stock. Although the eastern SSL stock was recently delisted, this information could identify residual or emerging threats and encourage future post-delisting monitoring through the stranding network. The use of the Northwest Region and Alaska Region stranding data allowed us to investigate and illustrate patterns in SSL stranding trends to better understand how characteristics of stranding incidents may change over space, and through time, and how stranding efforts may directly affect these changes. This may have implications for managers when assessing the costs and benefits of mitigating documented anthropogenic hazards to SSL populations (Chaloupka et al. 2008).

## Methods

### Animals and Study Area

Stranding data for (SSL) (*Eumetopias jubatus*) (n=1507) from 1990-2015 were obtained from the Level A stranding data from the Alaska Region (n=544) and the Northwest Region (n=963) Marine Mammal Stranding Networks. The data received from the Alaska Region (n=544) and the Northwest Region (n=963) included date of stranding, location of stranding, standard length of the sea lion (cm), sex, age class and details on signs of human interaction. The date of reported strandings were used as a proxy for time of death in dead stranded SSL (Flint et al. 2015), and the seasons were defined as summer (June-August), fall (September-November), winter (December-February) and spring (March-May) for summarizing trends. If

coordinates were not provided, the town closest to the stranding was used. Strandings occurred from as far northwest as St. Paul Island (57.1867° N, 170.2575° W) to as far south as Medford, Oregon (42.3265° N, 122.8756° W), encompassing both eastern and western SSL stocks. The eastern and western SSL stocks are federally recognized as being separated at Cape Suckling, 144° West longitude (Figure 1) (Allen and Angliss 2013). We compared data obtained from the Alaska Region, which included strandings from both stocks and the Northwest Region, which only contained strandings from the eastern SSL stock. Differences in Level A report content and effort to monitor strandings across both regions prevented this study from comparing stranding data between SSL stocks.

## Biometrics and Carcass Condition

Age class (n=1120), sex (n=955), and standard length (n=969) were documented for the majority of strandings, but decomposition of some carcasses prevented us from obtaining these data for all samples. One case was excluded from the Northwest Region stranding records because it occurred outside of US boundaries (i.e. British Columbia, Canada). Age class was categorized into five categories in each region, including pups, yearlings, sub-adults, adults, and unknown in the Northwest Region; and fetuses, pups, sub-adults, adults and unknown in the Alaska Region. Fetal cases were not identified as a category in Level A reports, which is why this category is excluded in the Northwest Region. In the Alaska Region, yearlings were identified as sub-adults because it is less/accurate to specify age class as yearling when considering stranding reports are only an index of a sighting (Personal communication, Kate Savage). For this reason, yearlings were also grouped with sub-adults in the Northwest Region for all analyses. The Alaska Region contains fetal cases solely as a result of more specific Level B stranding reports submitted by Alaska Department of Fish & Game, and therefore, were

included in this dataset. Sex was categorized as female, male or unknown by observing external genitalia. Carcass standard length was measured from the tip of snout to the tip of the tail and is provided in centimeters, or otherwise converted to centimeters for analysis. Carcass condition, when known, was defined as alive, fresh dead, moderate decomposition, advanced decomposition and mummified following the guidelines of Geraci and Lounsbury (2005). All carcasses are assigned to one of the five categories upon initial examination if able to be determined.

The Northwest Region used the age class defined in the NMFS examiners guide (NOAA 2017):

> "Pup/Calf: Animal is smaller than yearling size, or in a population where it would be younger than one-year-old.
>
> Yearling: Animal is judged to be approximately one-year-old, using length or time of year.
>
> Subadult: Animal is judged to be greater than one-year-old, but not yet mature
>
> Adult: Animal is judged or found upon necropsy to be sexually mature.
>
> Unknown: Unable to determine age"

### Signs of Human Interaction and Contributing Factors to SSL Mortality

Signs of human interactions were categorized for all cases, and placed into the following three categories: yes, no, or could not be determined. Human interaction do not imply cause of stranding or death, however, certain signs of human interaction were considered contributing factors to morality, including boat collision, gunshot wounds, fishery interaction, and other

human interaction (i.e. "ingested plastic, debris entanglement, wounds from other weapons, non-boat vessel related injuries, mutilation, etc.; NOAA 2017, pg. 16)." The data synthesized in this study provided very little information on other details of human interaction.

**Data Analysis**

### Biometrics and Carcass Condition

Pearson's Chi-squared test with Yate's continuity correction was used to determine significant differences in the proportion of carcasses that had unknown age class or sex between regions. The 1-sample proportion z-test with continuity correction was used to determine if there was a significant difference between proportions of male and female strandings between regions.

### Mapping and Temporal Trends

A geographic information system (ArcGIS 10.2.1, ESRI, Redlands, CA) was used to map all strandings. An adjusted scale and corresponding markers were used to document strandings using the best available information on location of stranding occurrence. Temporal trends were illustrated in a line chart using the annual ± SE number of strandings summed across all months each calendar year. Box plots were used to illustrate seasonal patterns in each region (RStudio 0.99.903; RStudio Team 2015). Statistical analyses were conducted with R Version 3.4.0 (RStudio Team 2015).

A binary logistic regression model was used to determine if the probability of a stranding occurrence in the spring/summer (Mar-Aug) as opposed to fall/winter (Sept-Feb) differed based on the value of two categorical covariates: age class (pups, sub-adults and adults) and region (Alaska and Northwest Region). Five generalized linear models (GLM) were constructed to

represent all subsets of including/excluding the covariates age class and region. Akaike information criterion (AIC) was used for model selection.

### Stranding Effort

The present study attempted to normalize the stranding data by accounting for effort following the distribution of the John H. Prescott Marine Mammal Rescue Assistance Grant Program in 2000. The funds were disseminated along US coastline waters for the purpose of recovery or treatment of marine mammals, the collection of data from living or dead stranded marine mammals for health research, and facility operation costs (MMHSRP 2010). An over dispersed Poisson regression model was used to compare the number of stranding reports between regions before and after the Prescott grant was awarded. Furthermore, Prescott grant recipients changed following 2010, and similar data from 2011-2015 were unable to be obtained for the purpose of this study.

## Results

### Biometrics and Carcass Condition

The proportion of male strandings in both regions was significantly greater than female strandings ($p < 0.0001$, Table 1). The Alaska Region exhibited a higher percentage of carcasses defined as unknown sex at 56% (n=307) in comparison to the Northwest Region, of which 25% (n=245) of cases were defined as unknown sex ($x^2 = 142.52$, d.f = 1, $p < 0.0001$, Table 1). Of the carcasses identified as unknown sex in the Alaska Region, 40% were in a state of moderate or advanced decomposition upon initial sighting and 27% of them were identified as alive perhaps accounting for difficulty in identifying sex of the stranded animal. Of the 307 cases identified as unknown sex, 223 individuals were also classified as unknown age class in the

Alaska Region. Age class categories varied across regions (Table 2). However, for cases where age class was determined, adults were the highest reported stranding age class. The Alaska Region had a higher percentage of unknown age classes at 49% (n=265) in comparison to the Northwest Region with 13% (n=122; $x^2$ = 191.79, d.f = 1, p < 0.0001, Table 2). Adults accounted for 54% of stranding cases in the Northwest Region. Due to inconsistency in age classes identified between the regions, as well as varying sample sizes, inter-regional comparisons of standard length across each age class were not possible. There was great variation across all age classes, which was supported by the high standard deviations in mean lengths, with exception of the fetal age class (Table 2).

The initial condition upon examination of the stranded SSL varied by region (Table 3). In Alaska, the initial condition of the majority of strandings was alive (24%), fresh dead (24%), or in a state of moderate decomposition (22%). In the Northwest Region, 36% of carcasses were in a state of advanced decomposition and 27% of carcasses were in a state of moderate decomposition. Carcass condition upon initial examination is vital to the quality of information, and can be utilized to better understand other factors analyzed in this study. Carcasses identified as heavily decomposed can potentially inhibit the determination of certain factors such as age, sex, cause of death, size or human interactions.

## Maps

### Alaska Region

Stranding events in Alaska waters ranged from as far north and west as St. Paul, to as far east as Ketchikan (55.4785° N, -131.7803° W), and as far south as Umnak on the Aleutian archipelago (53.2238° N, -168.4319° W). There were 292 (54%) strandings reported from the

eastern SSL stock and 252 (46%) strandings reported within the western SSL stock. There was a variable distribution of strandings with the highest concentration of strandings occurring around the Gulf of Alaska (Fig. 1a). The areas of Juneau (58.3019° N, 134.4197° W), Glacier Bay (58.6658° N, 136.9002° W) and Gustavus (58.4133° N, 135.7369° W) had the highest concentrations of strandings overall (Fig. 1a). Kodiak (57.7900° N, 152.4072° W) St. Paul (57.1225° N, 170.2799 ° W) and Seward (60.1042° N, 149.4422° W) had the second highest concentrations of strandings (Fig. 1a). The number of MMHSRP agreement holders and covered participants are highest in Southeast Alaska, with the second highest being in the southcentral which includes Seward and Kodiak region (NOAA 2016). Therefore, the spatial trends observed appear to be reflective of stranding effort distribution and human population size.

## Northwest Region

The Northwest Region represents a broad range of stranding locations with the most northern location being in the town of Point Roberts, Washington (48.5912° N, 123.0528° W) and the most southern stranding location located in the town of Brookings, Oregon (42.327° N, 124.1711° W; Fig. 2). The highest frequency of strandings was in Bandon Beach, Oregon with 89 strandings, or 9% of the total number of strandings occurring in the Northwest Region (Fig. 2). The second highest frequency of strandings occurred in Neah Bay, Washington, representing roughly 3% of the total number of stranding incidents. In the Northwest Region more strandings were detected on the Oregon coastline in comparison to the Washington coastline. Overall, 67.6% of all stranding reports occurred on the Oregon coastline, with 32.4% occurring on the Washington coastline. Despite the higher number of stranding reports in Oregon, Washington has more stranding agreement holders and covered participants in comparison to Oregon (NOAA

2016). The higher number of strandings in Oregon may be due to the higher number of haulouts and rookeries in Oregon compared to Washington.

## Temporal Analysis

### Alaska Region

Across all 25 years, the mean number of monthly strandings in the Alaska Region was 0.42 ± 0.11. There was no particular trend observed in the total annual mean number of strandings per month from 1990 to 2005 (Fig. 2). However, there was a slight increase in the mean number of monthly stranding reports from 2006-2010 and again from 2013-2015. The greatest spike in the mean number of monthly stranding reports was observed from 2013 to 2015 where the mean number of monthly strandings increased, although not significantly, from 2.33 ± 0.7 to 5.33 ± 1.9 ($W = 52.5$, $p > 0.05$).

The mean number of strandings across all seasons in the Alaska Region was 1.8 ± 2.3. Clear seasonal patterns were found in the Alaska Region across all years, with the highest stranding occurrences documented in the summer (max=21) and the lowest in winter (max=5; Fig. 3). The number of stranding reports in the spring and summer months was significantly higher than the number of stranding reports in the fall and winter months ($x^2 = 174.38$, d. f = 1, $p < 0.0001$). The winter season had a median of zero, and relatively low variation with a maximum number of stranding occurrences at 5 (Fig. 3). The spring season had the highest variation and a median of one. Winter and fall had the lowest interquartile ranges. Summer had the greatest interquartile range and highest maximum number of strandings reported at 21. Raw data indicated the highest total number of strandings across all twelve months occurred in July with

118 strandings and in June with 98 strandings reported. These high numbers explain the seasonal summer trend observed (Fig. 3).

As previously mentioned, the frequency of strandings across Alaska varied, with the highest concentration of strandings occurring in Glacier Bay and Gustavus in Southeast Alaska. More specifically, in Juneau, 45% of the strandings were reported to occur in the summer months. 82% and 74% of the strandings reported in Glacier Bay and, Gustavus, respectively occurred in the summer season.

## Northwest Region

The mean number of monthly strandings over the 25-year time period in the Northwest Region was 3.10 ± 0.4. Similar to the Alaska Region, there was no particular trend observed in the mean strandings per month from 1990 to 2002. After 2002, a slight increase in mean number of annual strandings occurred (Fig. 2). The increase in mean number of monthly strandings from 1.83 ± 1.75 in 2005 to 10.25 ± 2.63 in 2007 was not a result of a mass strandings ($W = 2$, $p < 0.05$).

The mean number of strandings across all seasons in the Northwest Region was 3.1 ± 3.9. The Northwest Region revealed clear seasonality trends, with a high number of stranding occurrences in the spring and summer seasons (Fig. 4). The number of strandings reports in the spring and summer months was significantly higher than the fall and winter stranding reports ($x^2 = 103.04$, d. f = 1, $p < 0.0001$). The winter and fall seasons had a median of one, with low variation. When comparing fall and winter, fall had the highest interquartile range. Raw data indicated the highest number of stranding occurrences, with a maximum of 149 stranding occurrences in July and 111 strandings reported in August across the time series. These high

numbers occurred in the summer months and explain the seasonal summer trend observed (Fig. 4).

There was a significant difference in the proportion of strandings that occurred in spring and summer as opposed to fall and winter between the Alaska Region (81%) and the Northwest Region (67%) ($p < 0.05$). However, there was no significant difference between the proportions of pups, sub-adults and adults stranding in spring and summer ($p > 0.05$). The proportion of stranding reports in spring and summer for pups (73%), sub-adults (68%) and adults (69%) was relatively close in value. AIC analyses suggested the model including the region covariate alone was the best model and carried 57% of the model weight (Table 4).

## Signs of Human Interaction

Findings of human interaction varied across regions (Table 5). Human interaction accounted for 17% in the Northwest Region and 25% in the Alaska Region where signs of human interaction include boat collision, gunshot, fishery interaction and other human interaction, as previously defined (Table 5). A smaller proportion exhibited signs of other human interaction at 2% in the Northwest Region and <1% in the Alaska Region. Overall, signs of human interaction could not be determined for the majority of cases (Alaska: n=339; Northwest: n=681). 70% of these cases in the Northwest Region and 50% of these cases in the Alaska Region were categorized in a state of moderate or advanced decomposition. Other detailed pathologic findings included in Level B and C reports that would provide more information on signs of human interaction were limited because necropsies were only conducted on roughly 26% of reported SSL stranding events across both regions. Therefore, accounts of human interaction or factors contributing to the causes of strandings must be considered preliminary.

### Stranding Effort

The annual number of stranding reports was ~5 times higher in the Alaska Region and ~8 times higher in the Northwest Region after the Prescott grant was awarded in 2001 ($p < 0.05$). However, the annual number of stranding reports did significantly differ after the Prescott grant was awarded ($p < 0.05$). There was insufficient evidence to suggest that the mean number of annual stranding reports significantly differed between regions before the Prescott grant was awarded ($p=0.805$). The effects of Prescott grants on the number of reported strandings did not differ significantly between the two regions ($p > 0.05$).

## Discussion

### Biometrics and Carcass Condition

Adult strandings occurred most frequently, with the exception of a large number of cases in the Alaska Region whose age classes were unidentifiable. Some of this discrepancy may be due to small Steller sea lions potentially being subjected to greater scavenging or movement of the carcass due to tides washing them away. This is contrary to the findings of a live pinniped stranding study, in which adult harbor seals (*Phoca vitulina)* and elephant seals accounted for a small proportion of total live strandings across all age classes (Colegrove et al. 2005). Elasticity analyses conducted by Maniscalco et al. (2015) and age-structured modeling (Holmes et al. 2007) indicate population growth rate is most sensitive to changes in adult survival, and therefore, the large proportion of adult strandings has the potential to cause impediments in recovery of the western SSL stock and recently delisted eastern SSL stock.

Males (n=392) in the Northwest Region stranded more frequently than females (n=326), and of the cases where sex was identified, males (n=176) in Alaska also stranded more

frequently than females (n=61). The proportion of male strandings was significantly higher than the proportion of female strandings reported in both regions ($p < 0.0001$, Table 1). This information is consistent with other studies that reported SSL males to have higher mortality rates across all age classes (Calkins et al. 1982). Shuert et al. (2015) also found the mean survival rate for female SSL to be slightly higher than males. In general, males have more extensive and variable movements (Raum-Suryan et al. 2002), which may help to explain the higher frequency of male strandings. Higher male strandings may also be attributed to higher nutritional requirements by larger males and male-male competition during active reproduction and time spent on rookeries (Clutton-Brock and Isvaran 2007; Hogg and Forbes 1997). For harbor seals, females stranded at a significantly higher rate than did male harbor seals (Colegrove et al. 2005). Although prior research findings provide support for observed stranding trends in sex and age class, Peltier et al. (2013) stresses the need for information on physical components that include processes which will determine carcass drift, including tides, currents and winds. This type of information would allow us to better interpret the current study findings.

## Spatial Analyses

Spatial analyses in Alaska showed the highest concentration of strandings occurred in Southeast Alaska, which is also where the highest number of agreement holders are located. The second highest concentration of strandings was in southcentral Alaska, again reflective of the second highest concentration of agreement holders of the Alaska Region. Although the distribution and number of stranding stakeholders in Alaska is not known for the entire time series, it was known for a substantial portion of the time series (2001-2010), including an increase of five stranding stakeholders in this region following 2010 (Personal communication, Kate Savage). The observed spatial trend is not surprising, but in many ways, may be

problematic. Although we would expect a lower number of strandings to occur in waters inhabited by the eastern stock due to their increasing population abundance at >3% per year since the 1970s (Pitcher et al. 2007) and more recent delisting of its prior 'threatened' status (Fritz et al. 2014), the uneven distribution of stranding stakeholders across Alaska influences the effort of finding stranded SSL, and thus may not reflect true SSL abundance and distribution. Due to differences in stranding effort distributed within the Alaska Region, the sample size for the western SSL stock was relatively low. This did not allow for comparisons between SSL stocks, but did allow for robust comparisons between the Alaska Region and the Northwest Region.

Spatial analyses in the Northwest Region differed from those observed in the Alaska Region. In the Northwest Region, spatial analyses did not appear to be influenced by stranding effort if considering the number of stranding stakeholders present in Oregon and Washington. A higher number of stakeholders were present in Washington, yet, nearly 68% of the Northwest Region stranding reports occurred on the Oregon coastline. This suggests increased effort did not result in increased number of stranding reports in Washington. The California current flows south from Washington through Oregon and may have contributed to the greater abundance of strandings in Oregon (NOAA 2016). In addition, there are no SSL rookeries in Washington which could account for the low occurrence of strandings as there would be less aggregations of SSL and pups in the summer. The high concentration of strandings occurring around Coos Bay, Oregon could be a result of increased anthropogenic interaction when considering Coos Bay has a large population center with an associated commercial fishing hub and perhaps, increased likelihood of stranding reporting (Lee 2016). The second highest concentration of strandings occurred along the coastal zone of Curry County, Oregon in relatively close proximity to

Pyramid Rock, Long Brown and Seals Rock, which are sites that were designated as critical habitat for SSL, and should be further monitored post-delisting for elevated number of strandings. Further investigation of distribution of prey persistence and correlated SSL movement patterns may help to better interpret the spatial trends observed in the Northwest Region (Womble et al. 2009).

## Temporal Analyses

Temporal analyses revealed the mean number of stranding reports increased following 2002. This increase is likely a direct result of the distribution of federal funds through the Prescott Awards, and not reflective of SSL population changes. The apparent steep incline in the mean number of annual stranding reports from 2005-2007 in the Northwest Region is difficult to interpret. Many of these carcasses were categorized as in a state of advanced decomposition causing difficulty in identifying potential signs of human interaction and in allowing carcass examination to determine possible causes of death.

Stranding occurrences can be associated with storm surges and El Niño events (Dunlap 1995; Greig et al. 2001), and prior studies have shown a correlation between El Niño events and higher number of California sea lion strandings (Greig et al. 2005). A weak El Niño did occur during the time period of increased SSL strandings (2006-2007), but likely had little effect on food availability considering it was a weak event. Therefore, the cause of the increase in stranding events remains unknown. Furthermore, during the time of a moderate El Niño from 2009-2010, there was no apparent increase in mean number of annual SSL strandings, suggesting weak and moderate El Niño events have little influence on SSL strandings observed in these datasets. Future research on other oceanographic and atmospheric anomalies that may have attributed to the temporal trend observed here are encouraged.

Additional temporal analyses indicated clear seasonal patterns, with higher stranding occurrences in the summer months (June-August) across both regions. These findings are consistent with Lee (2016) who also identified the highest number of SSL strandings to occur in July and August for years 2006-2014 in the Pacific Northwest. Based on the GLM analyses done in the present study, the proportion of strandings in spring and summer was significantly higher in the Alaska Region versus the Northwest Region suggesting region may account for the observed seasonality variation ($p < 0.05$). This may be explained by geographic differences between Alaska and the Northwest Region. If we consider the expansive coastlines in Alaska and lower ability to have coverage year round in comparison to Washington and Oregon, we would suspect a higher concentration of strandings to be reported in Alaska in the spring and summer. In the Northwest Region, milder climates and higher, more equally distributed populations inhabiting coastal areas allows for more consistent stranding effort to occur during three seasons (spring, summer, fall) or year-around.

## Signs of Human Interaction

Signs of human interaction couldn't be determined for 50% of cases in the Alaska Region and 70% of cases in the Northwest Region. This is likely a direct result of the condition many of these carcasses were in upon initial examination making it difficult to observe signs of human interaction. As in other studies, with such a high number of cases identified in a moderate or advanced state of decomposition, it is difficult to determine cause of death (Koch et al. 2013).

Across the entire 25-year time period of the study, a large proportion of human interaction cases, (i.e., 81%) were identified between 2008 - 2015 in both regions. These cases include boat collision and gunshot wounds, with many of these cases reported following the year 2006. It is difficult to ascertain reasons for this increase in reporting other than it being a result of

increased effort and public awareness. However, one notable result was 40% of the cases identified as being shot occurred within 2015, and a high proportion (88%) of them occurred during the summer and spring months in one particular area. More detailed Level B reports were obtained from ADF&G and revealed seven of the eleven cases to be considered part of a group event that occurred in Cordova, near the Copper River commercial salmon drift gillnet fishery. Five of these cases were males, and four cases identified as adults and three cases as juvenile/subadults. In that same year, a climate anomaly was documented, also known as the marine heat wave (Bond et al. 2015; Di Lorenzo and Matua 2016). This has been identified as a causative agent of a major shift in the forage food base (Personal communication, Shannon Atkinson) and subsequent changes in prey availability and distribution, which may have affected SSL behavior and resulted in increased numbers of SSL targeting fishing nets.

## Continued Utilization of Stranding Database and Implications for Conservation

Level A stranding reports were not designed to elucidate cause-specific mortality trends. The degree of confirmed biometric data included in the present study is associated with varying levels of uncertainty. Inability to distinguish between cases associated with low, moderate or high levels of effort hampers a statistically and scientifically robust study from having strong conclusions resulting from these data. Another caveat associated with these data involves stranding response effort and inconsistencies in this effort across time and space. When considering the amount of funding supporting stranding networks, the need for refinement and standardization of forms is recommended.

As revealed in other studies, an apparent increase in strandings and subsequent reporting may be directly linked to increased observational effort, and not an accurate representation of a true increase in strandings (Berrow and Rogan 1997, Evans et al. 2005, Danil et al. 2010). In

order to make any definitive, conclusive statements in regards to Level A data, we must have a better measurement of effort. Level B and C reports produced from post-mortem examinations serve great value when attempting to identify cause-specific trends. However, only 26% of the cases in the present study had necropsies performed, despite the ESA (Endangered Species Act) listing. Studies such as the present one would benefit from stranding networks organizing the data to account for annual effort and consistent reporting templates used to collect Level B and Level C data to minimize uncertainty. If successful, researchers would be able to synthesize data and determine stranding patterns with as little bias as possible. It is recognized that there is substantial difficulty in doing this considering the vast size of Alaska and small human population in many parts of the state, and the role limited funding may play.

For the purpose of conservation efforts and the desire to utilize stranding data to inform management of SSL, the present study has a few specific recommendations. We stress the need for increased number of stranding response participants in western Alaska. This would allow for minimization of bias when attempting to analyze temporal and spatial trends in SSL strandings. The western SSL stock that inhabits waters west of Samalga pass (~170 ° W) is in a state of decline, and reasons for this are not clear (Fritz et al. 2013). This area of concern has limited information available and associated unknown causes of continuous decline of western SSL inhabiting that area. Annual field effort should prioritize the collection and analysis of stranded SSL across the geographic range. Due to the limited human population west of Samalga Pass, and minimal stranding response timing in that area, it is unlikely stranding data will be informative to management plans without improvement in surveillance efforts. In contrast, stranding effort in the Northwest Region is more uniformly distributed and likely representative of the eastern SSL stock population distribution. Our study has highlighted the need for

supporting marine mammal stranding monitoring and surveillance programs while also elucidating a clear need to improve and standardize current data collection and reporting processes. Continued and improved stranding surveillance programs that will support increased post-mortem examinations are warranted. With improved surveillance and quality of stranding data, researchers can better understand both short-term and long-term factors affecting SSL mortality.

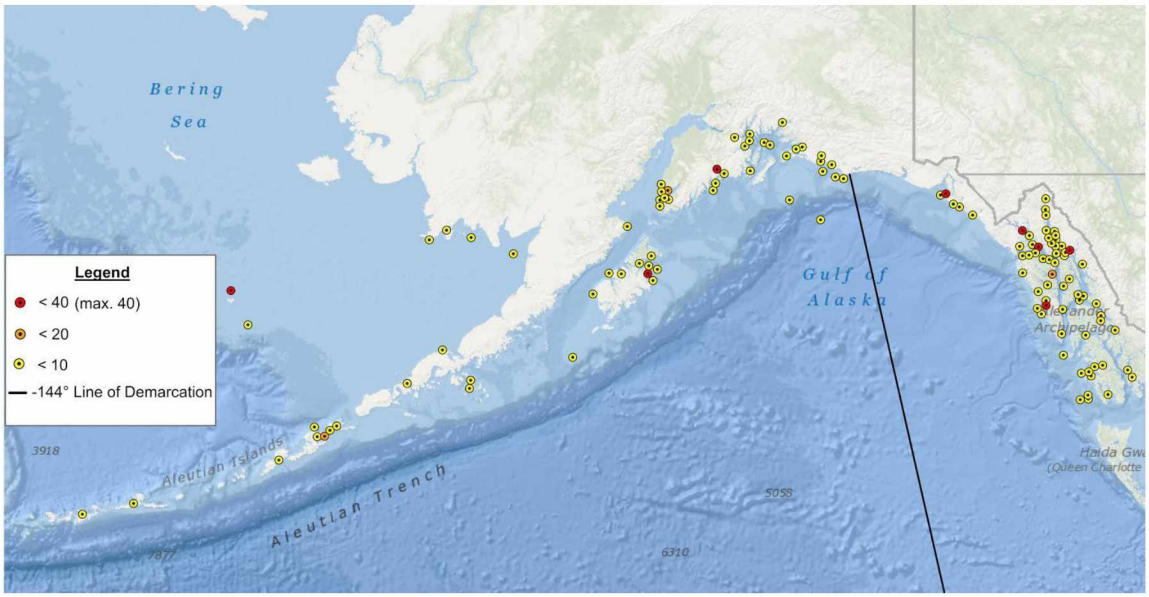


**Fig. 1a.** Study area map for the Alaska Region, encompassing both eastern and western Steller sea lion (SSL) stocks, in the Bering Sea and Gulf of Alaska. Markers identify stranding locations and colors indicate areas of higher and lower density strandings. The study area includes stranding incidents from as far Northwest as St. Paul Island (57.1867° N, 170.2575° W) and as far southeast as Ketchikan (55.47850° N, -131.78033° W). The 144 degree line longitude separates the two stocks of SSL.

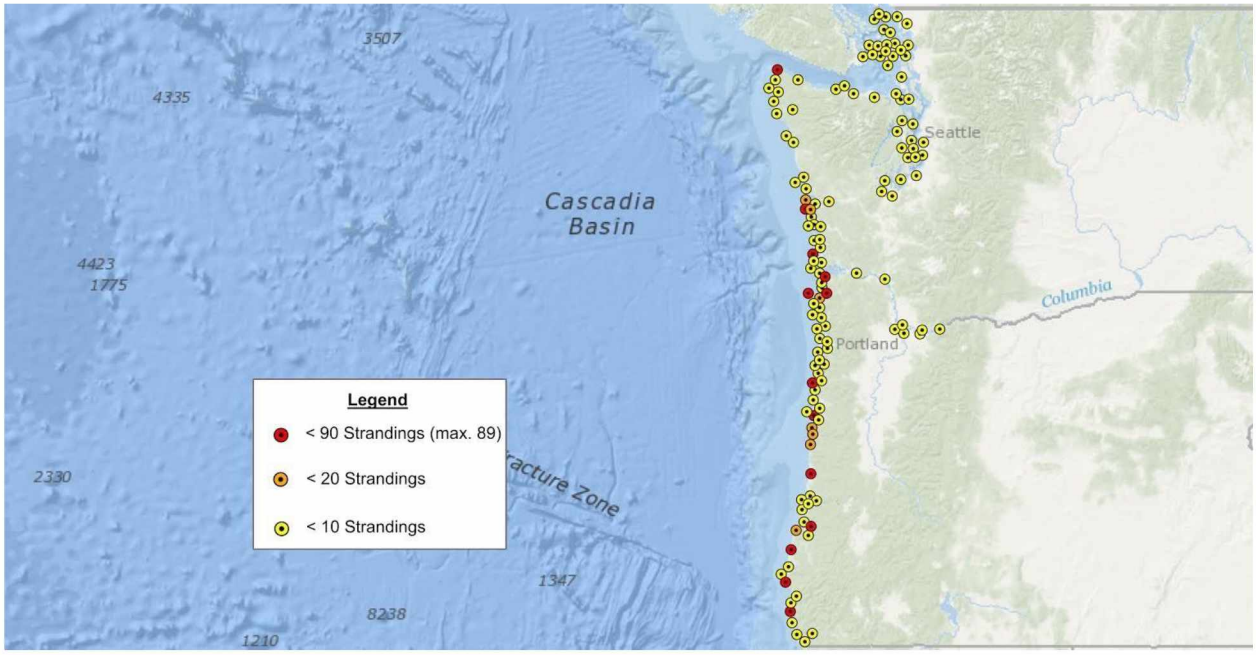


**Fig. 1b.** Study area map, including a portion of the eastern stock of the Steller sea lion population that extends from Northern Washington down to Southern Oregon. Markers identify stranding locations and colors indicate areas of higher and lower density strandings over the entire time 25-year time period (1990-2015). The study area includes stranding incidents from as far North as Point Roberts, Washington (48.5912° N, 123.0528° W) as far south as far south as Brookings, Oregon (42.327° N, 124.1711° W).

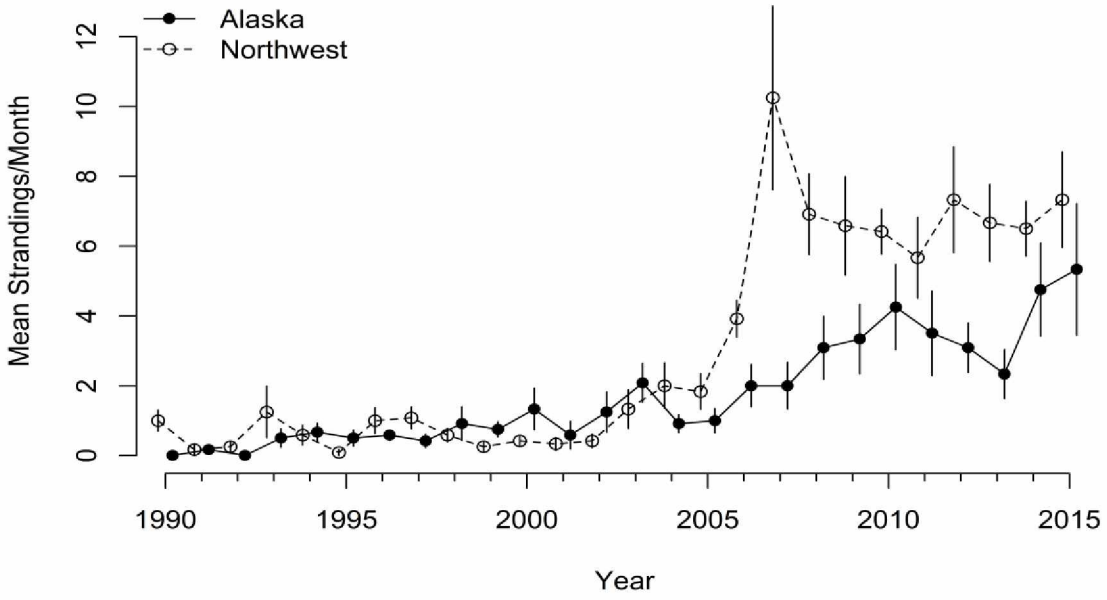


**Fig. 2.** The mean ± SE reported number of strandings across all months per year including 1990-2015. These data encompass the Alaska region (solid circles), which includes stranding incidents from the Gulf of Alaska and the Bering Sea, as well as strandings from the Northwest region (open circles), which includes strandings in the eastern Pacific Ocean, along the coast of Oregon and Washington. Data illustrated are not corrected for surveillance and stranding effort.

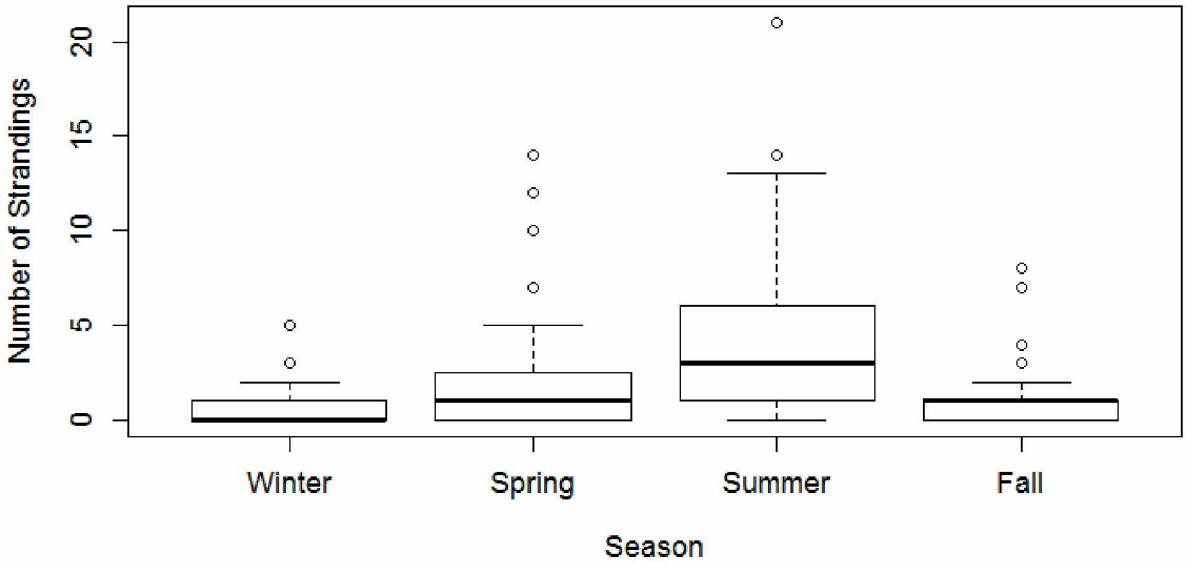


**Fig. 3.** The boxplot displays the variability in the total number of stranding events occurring each season over a 25-year period from 1990-2015 in Alaska. These data include Steller sea lion (pups through adults) stranding incidents occurring in the Alaska Region, encompassing both the eastern and western SSL stocks. Values below the lower whisker represent the lowest 2.5% of all values, and the highest 2.5% of values are found above the upper whisker of the box plot. 25% of the values fall below the 1st quartile, or lower end of the box, while 75% of the values fall below the 3rd quartile, the upper end of the box. The median is represented by the horizontal line, within the 1st and 3rd quartile. Outliers are illustrated as open circles above the 3rd quartile.

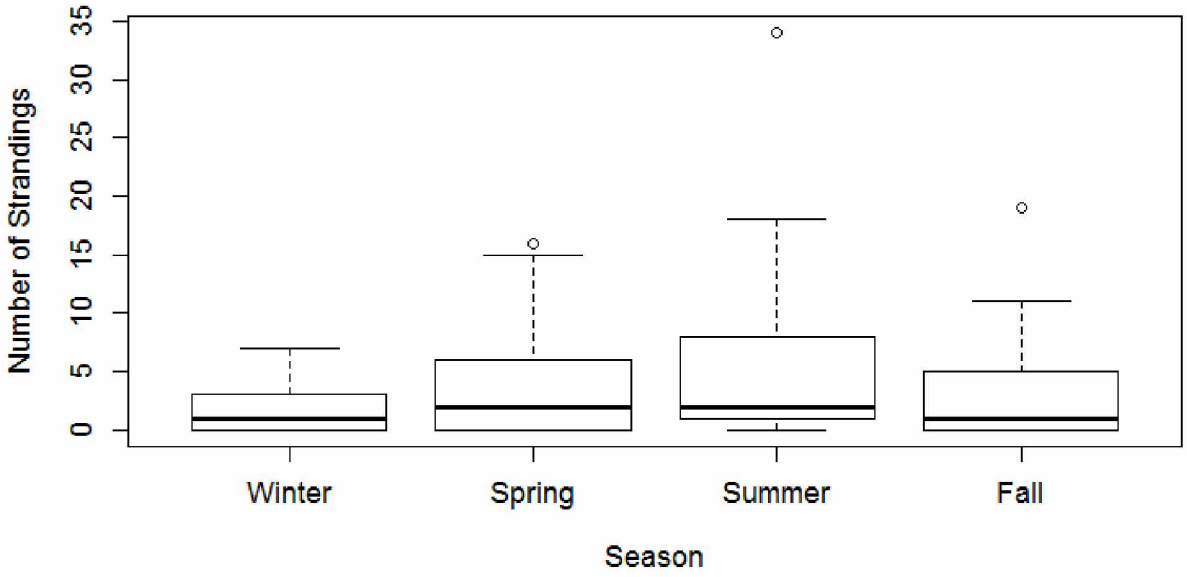


**Fig. 4.** The boxplot displays the variability in the total number of stranding events occurring each season over a 25-year period from 1990-2015 in the Northwest Region. These data include Steller sea lion (SSL); live pups through adults stranding incidents occurred in the Northwest Region, including the Oregon and Washington coastline. Values below the lower whisker represent the lowest 2.5% of all values, and the highest 2.5% of values are found above the upper whisker of the box plot. 25% of the values fall below the 1st quartile, or lower end of the box, while 75% of the values fall below the 3rd quartile, the upper end of the box. The median is represented by the horizontal line, within the 1st and 3rd quartile. Outliers are illustrated as open circles above the 3rd quartile.

**Table 1.** The percentages and sample size of each sex from stranded Steller sea lion (SSL) occurring in each region, AK (Alaska) and Northwest (NW) Regions. The percentages displayed represent the proportion of male, female and unknown of the total stranding incidents occurring per region. *Significant difference from females in each region ($p < 0.0001$).

| **Sex** | **Percentage of AK Stranding Incidents** | **Percentage of NW Stranding Incidents** |
|---|---|---|
| Unknown | 56.4% (n=307) | 25.4% (n=245) |
| Female | 11.2% (n=61) | 33.7% (n=326) |
| Male | 32.4% (n=176)* | 40.7% (n=392)* |
| **Total Number of Stranded Steller sea lions** | **544** | **963** |

**Table 2.** The proportion of stranding incidents of Steller sea lion (SSL) in each age class and their mean standard lengths (cm, $\bar{x} \pm$ SD) occurring in the AK (Alaska) and NW (Northwest) regions. Note that the age classes are defined slightly differently in each region.

| **Age Class** | **Number of AK Stranding Incidents** | **AK Mean Length (cm) ± SD** | **Age Class** | **Number of NW Stranding Incidents** | **NW Mean Length (cm) ± SD** |
|---|---|---|---|---|---|
| Fetus | <1% (n=4) | 78 ± 0.00 | Pup | 14.1% (n=136) | 100.6 ± 17.3 |
| Pup | 4.6% (n=25) | 89.4 ± 24.6 | Yearling | 6.9% (n=66) | 127.9 ± 19.1 |
| Sub-Adult | 13.9% (n=76) | 187.0 ± 46.0 | Sub-Adult | 12.5% (n=120) | 191.2 ± 41.8 |
| Adult | 31.9% (n=174) | 265.3 ± 53.7 | Adult | 53.8% (n=519) | 241.7 ± 51.3 |
| Unknown | 48.7% (n=265) | NA | Unknown | 12.7% (n=122) | NA |
| **Total** | **544** | **NA** | **NA** | **963** | **NA** |

**Table 3. Percentage of** Steller sea lion (SSL) carcass condition at examination in the Alaska (AK) and the Northwest (NW) Regions.

| **Initial Condition** | **Percentage of AK Stranding Incidents** | **Percentage of NW Stranding Incidents** |
|---|---|---|
| Alive | 23.7% (n=129) | 9.1% (n=88) |
| Fresh Dead | 24.2% (n=132) | 20.7% (n=199) |
| Moderate Decomposition | 22.2% (n=121) | 27.1% (n=261) |
| Advanced Decomposition | 16.4% (n=89) | 35.9% (n=346) |
| Mummified | 3.9%(n=21) | 2.7%(n=26) |
| Condition Unknown | 9.6% (n=52) | 4.5% (n=43) |
| **Total Number of SSL Stranding Incidents** | **544** | **963** |

**Table 4.** Five generalized linear models (GLM) were constructed to represent all subsets of including/excluding the covariates age class and region, where p is the probability of a stranding occurring the spring/summer as opposed to fall/winter for region $i$. Akaike information criterion (AIC) was used for model selection.

| **Model** | **AIC** | **Δ AIC** | **AIC Weight** |
|---|---|---|---|
| logit(p) = $B_0$ + $B_1$ * Region | 1351.94 | 0 | 0.57 |
| logit(p) = $B_0$ + $B_1$ * Region + $B_2$ * Age | 1353 | 1.06 | 0.33 |
| logit(p) = $B_0$ + $B_1$ * Region + $B_2$ * Age + $B_3$ * Region * Age | 1355.43 | 3.48 | 0.1 |
| logit(p) = $B_0$ | 1372.69 | 20.75 | 0 |
| logit(p) = $B_0$ + $B_1$ * Age | 1375.1 | 23.16 | 0 |

**Table 5.** Steller sea lion (SSL) human interaction cases in the Alaska (AK) and the Northwest (NW) Regions

| **Human Interaction Type** | **AK Region Stranding Incidents** | **NW Region Stranding Incidents** |
|---|---|---|
| Boat Collision | <1% (n=4) | <1% (n=4) |
| Fishery Interaction | 20.7% (n=113) | <2.70% (n=26) |
| Gunshot | 5.1% (n=28) | 12.7% (n=122) |
| Other | <1% (n=2) | 2.18% (n=21) |
| **Total Number of Human Interaction incidents** | **147** | **173** |
| **Total Number of Stranding Incidents** | **544** | **963** |

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Chapter 2: Role of Pathogens in Steller sea lion (*Eumetopias jubatus*) Abortions, Premature Births and Neonatal Death[2]

Abstract

Steller sea lions (SSL; *Eumetopias jubatus)* experienced a decline in abundance in the 1960s, which led to the listing of the western SSL stock as endangered and the eastern SSL stock as threatened under the US Endangered Species Act. A decline in the estimated birth rates for adult females in the western stock from the 1970s to the mid-1980s suggests reproductive failure may have contributed to a decline in abundance. The ability of pathogens to affect reproductive health and population stability warrants further investigation on the role pathogens may play in SSL spontaneous abortions, premature births and neonatal deaths. We utilized archived lung, skin lesion and placenta tissues from SSL fetal carcasses (n=20) that were collected in Alaska from 1998 to 2015, along with one fetal carcass collected from Washington. Tissues were tested by PCR for *Coxiella burnetii, Brucella* spp., *Chlamydophila* and all species in the genus morbillivirus. Gross necropsy and histology findings were also summarized in the context of the PCR findings. Von Bertalanffy growth curves were used to estimate fetal (n=16) and neonatal weight (n=2) (kg), curvilinear length (CL; cm) and straight length (SL; cm) as a function of month.

Locations of carcass sightings in Alaska represented both SSL stocks. Age classifications varied, and included 35% aborted fetuses, 45% premature pups, 10% neonates and 10% intrauterine fetuses. Analysis of tissues for *Chlamydophila* and *C. burnetii* DNA using polymerase chain reaction (PCR) were uniformly negative. Three lung tissue samples were positive by PCR and one case was suspect for the IS711 *Brucella* target sequence. An attempt to

[2] Esquible JA, Burek-Huntington K, Atkinson S, Bortz E, Goldstein TA, Beckmen K and Pabilonia K. 2018. Prepared for submission to Journal of Disease of Aquatic Organisms

culture *Brucella* was unsuccessful. This study is the first to detect *Brucella* spp. bacterial DNA by PCR in SSL lung tissues. Two skin lesion samples and one placenta were positive by PCR for Phocine Distemper Virus (PDV). Both skin lesions that were positive for PDV had vesiculoulcerative dermatitis and the freshest lesion contained inclusion bodies upon histologic examination, and thus may be a result of the PDV infection. We highlight the need for continued disease surveillance programs to improve our understanding of the pathogenicity of these infectious disease agents in Alaskan waters.

**Introduction**

Steller sea lions (SSL) (*Eumetopias jubatus)* have been intensively studied as a result of the major decline in abundance beginning in the 1960s, which led to the listing of the western SSL stock as endangered, and the eastern SSL stock as threatened under the US Endangered Species Act (Loughlin and York 2000; Ferrero and Fritz 2002; Miller et al. 2005; Atkinson et al. 2008). The eastern stock was delisted in 2014; however, scientists are still attempting to determine causes of differences in regional trends (Fritz et al. 2013). Disease, malnutrition, predation, climate change, decreased birth rate, entanglement in marine debris and other factors may have contributed to the decline (Loughlin 1998; Pitcher et al. 1998; Trites and Donnelly 2003; Burek et al. 2005; Atkinson et al. 2008). A decline in estimated birth rates for adult females in the western stock from the 1970s to the mid-1980s suggests reproductive failure may have contributed to a decline in abundance (Pitcher and Calkins 1981; Calkins and Goodwin 1988). Age-structured modeling by Holmes et al. (2007) suggests the birth rate in the central Gulf of Alaska had continued to decline through 2004 and declining birth rate may be problematic for the recovering western SSL stock. Poorly documented accounts of multiple dead

pups at discreet locations suggests that at least occasional and unpredictable occurrences of perinatal mortality have taken place.

Female SSL typically give birth from mid-May to mid-June, and breed about 10 days afterwards (Pitcher et al. 1998). Females exhibit embryonic diapause and thus delayed implantation for three months, in which the blastocyst attachment can occur between late September and October (Pitcher and Calkins 1981). Winship et al. (2001) suggests there is very little fetal growth occurring prior to February. Gestation can last for up to 11.5 months (Pitcher and Calkins 1981). Pitcher et al. (1998) observed abortion during the last 4.5 months of gestation. Considering the previous findings on SSL reproductive biology, we suspect the time of carcass collection in the present study is rather close to the time of abortion, premature birth and neonatal death. Although the primary focus of this study was to elucidate the role reproductive pathogens may play in reproductive failure, we also illustrate fetal growth curves that provide a reasonable representation of the varying sizes of SSL fetuses from February-July.

*Coxiella burnetti,* a zoonotic bacteria and reproductive pathogen, is widely recognized for causing "Q fever" in domestic mammals. The infected mother can transmit the disease via shedding in the placenta during parturition (Maurin and Raoul 1999). Chronic infection with C. *burnetii* may lead to abortion, premature birth, dead or weak offspring in cattle, sheep and goats (Lang 1990; Arricau-Bouvery and Rodolakis 2005; Rousset et al. 2008). In 1998, C. *burnetii* placentitis was reported in an ill, pregnant Pacific harbor seal (*Phoca vitulina richardsi)* in Northern California (Lapointe et al. 1999). In 2008, it was detected again in the placenta of a dead pregnant SSL stranded off the Washington coastline, which expanded the geographic range of *C. burnetii*; it is uncertain whether the pathogen contributed to the mammal stranding or death (Kersh et al. 2010). More recent molecular and serological studies confirm that the western SSL

stock and northern fur seal (*Callorhinus ursinus)* populations in Alaska are commonly exposed to and get infected with *C. burnetii* (Minor et al. 2013). Researchers have yet to elucidate implications of *C. burnetii* on marine mammal health, considering its novel discovery in marine mammals and lack of association with abortion.

Species of the genus *Brucella* are widespread zoonotic bacteria in the marine environment (Nymo et al. 2011; Guzmán et al. 2012; Hernández-Mora et al. 2013) and known to cause abortion in many wildlife and domestic animals (Ewalt et al. 1994). *Brucella* spp. have also been found in a variety of marine mammals with an unclear role in abortion and disease (Foster et al. 2002, Hoover-Miller et al. 2017). Transmission varies by case, is not limited to sexual activity, and can include vertical transmission, physical trauma and ingestion during feeding (Foster et al. 2002). Brucellae was first isolated from marine mammals including a stranded harbor seal (*Phoca vitulina),* harbor porpoise (*Phocoena phocoena)*, common dolphins *(Delphinus delphis),* and an aborted fetus of a bottlenose dolphin in 1994 in the United States (Ewalt et al. 1994; Ross et al. 1994; Ross et al. 1996). Burek et al. (2005) conducted a serosurvey in Alaska and found one SSL seropositive for antibodies to *B. abortus* and 195 that were seronegative, and Nymo et al. (2018) detected *Brucella* antibodies in two SSL. The zoonotic ST27 strain of *Brucella* has been detected by PCR in three placentae and fetal tissues in California sea lions (Whatmore et al. 2017), with evident transplacental transmission (Goldstein et al. 2009). Two of these placentae were also culture positive and exhibited signs of inflammation and multifocal acute necrosis (Goldstein et al. 2009). Duncan et al. (2014) detected B. *pinnipedialis* in northern fur seal placentas, with one placenta exhibiting severe placentitis (Duncan et al. 2014). Harbor seals in Alaskan waters had substantial portions of the population that tested positive in a recent *Brucella* serology study (Hoover-Miller et al. 2017); however

pathology consistent with *Brucella* infection is absent in phocid seals (Nymo et al. 2011). Pathology has been exhibited in the placentae of a limited number of otariid seal species, whereas in phocid seals reproductive pathology has no association with *B. pinnipedialis* infection and vertical transmission of this species of *Brucella* has not been observed (Nymo et al. 2011), suggesting pathogenicity of *Brucella* in phocid seals versus otariid seals may be different. Researchers have yet to isolate the zoonotic ST27 strain in Alaskan waters (Nymo et al. 2018) or in SSL, which is of great interest when considering this strain is zoonotic and could be associated with public health risks. The present study used PCR to detect *Brucella* spp.

Members of the family Chlamydiaceae include two genera, *Chlamydophila* (*C.*) and *Chlamydia* (*Cp.*), which encompass several species including *Cp. trachomatis, Cp. suis, Cp. muridarum,* C. *abortus,* C. *caviae,* C. *felis,* C. *pecorum,* C. *pneumoniae* and C. *psittaci* (Kauffold et al. 2014). Prior to the reclassification of *Chlamydiaceae* into two genera *Chlamydia and Chlamydophila* in 1999, *C. abortus* was formerly known as *Cp. psittaci* and *C. pecorum* was formerly known as *Cp. pecorum* (Everett et al. 1999; Berri et al. 2009). These members cause reproductive abortions, respiratory disease, and cardiovascular disease in a wide range of organisms (Beeckman and Vanrompay 2009). Transmission varies by species and may include horizontal transmission via ingestion or inhalation and venereal transmission (Loock et al. 2003; Kauffold et al. 2014). *Chlamydophila abortus* has been the causative infectious agent of ovine enzootic abortion in sheep and reproductive disorders in other large ruminants (Vidal et al. 2017; Rodolakis et al. 1998; Longbottom and Coulter 2003). *C. psittaci* infections are rather common in birds and have affected 465 species (Kaleta and Taday 2003), and it is recognized as a zoonotic pathogen. *C. psittaci* has caused respiratory outbreaks affecting the US turkey industry and an epidemic in the US poultry industry in the 1950s and 1960s (Beeckman and Vanrompay

2009; Meyer 1967). Serological studies report *C. psittaci* antibody presence in SSL (Burek et al. 2005), Hawaiian monk seals (*Monachus schauinslandi)* (Aguirre et al. 2007) and an Atlantic bottlenose dolphin (Schaefer et al. 2009). There has been one report of an isolation of C. *psittaci* in an aborted SSL fetal lung by Spraker and Bradley (1996). Schaefer et al. (2009) suggests shedding by local bird populations may be the explanation for *C. psittaci* seroprevalence in dolphins; however, disease transmission and implications for reproductive health in SSL are still unknown.

The genus *morbillivirus* is composed of many species including phocine distemper virus (PDV), canine distemper virus (CDV), cetacean morbillivirus (CeMV), measles virus, peste-des-petits-ruminants virus and rinderpest virus. The general mode of transmission for morbillivirus is through horizontal transmission by direct contact and through ocular, oral or respiratory fluids that contain the virus (Williams 2001; Appel et al. 1981). Typically, severe pneumonia and necrosis of the bronchial and bronchiolar epithelium as well as neurological signs including convulsions, seizures and head trauma are consistent with morbillivirus infection (Rowles et al. 2011; Duignan et al. 2014). In PDV and CDV, initial viral replication likely occurs in the lymphoid cells with further spreading to epithelial and endothelial cells (De Swart et al. 2007; Appel 1969; Krakowka et al. 1980). In pinnipeds, PDV was first documented in 1988 as causing an epidemic in European harbor seals (*Phoca vitulina vitulina)* and gray seals (*Halichoerus grypus)* (Osterhaus et al. 1990; Heide-Jørgensen et al. 1992). In 2002, PDV was identified as the causative agent of an epizootic in harbor and gray seals in Europe (Jensen et al. 2002; Barrett et al. 2002; Härkönen et al. 2006). In 2006, PDV was isolated from tissues of some of the harbor seals, gray seals and hooded seals (*Cystophora cristata)* involved in an unusual mortality event off the coasts of Maine and Massachusetts (Earle et al. 2011). CeMV has also contributed to

widespread epizootic and mortality events in cetaceans and dolphins (Bossart et al. 2010; Domingo et al. 1990; Duignan et al. 1996; Lipscomb 1994). In 2012, an "abortion storm" occurred off the coast of Kodiak and resulted in four SSL abortions in which disease was suspected and PDV was detected by PCR (Burek-Huntington K, personal communication September 1, 2015). Given that pinniped females infected with PDV may be prone to abortion (Duignan et al. 2014), further investigation on SSL aborted fetuses could provide novel insights into the potential prevalence and pathogenicity of PDV in SSL spontaneous abortions.

The present study utilizes archived tissue samples from SSL aborted fetuses, premature pups, neonatal pups and one intrauterine fetus in efforts to detect the presence of C. *burnetti, Chlamydophila spp., Brucella* spp. and morbilliviruses using PCR for the bacterial species and RT (reverse transcriptase)-PCR for morbillivirus. Data from gross necropsy reports and histopathology reports were summarized to identify any abnormalities and attempt to associate PCR findings with pathology. The present study fully recognizes the possibility of several etiologies of spontaneous abortions and premature births. Utilizing basic molecular techniques for detection of infectious disease agents and determining if any of these findings correlate with data from the gross necropsy and histopathology reports may provide insight into the role infectious disease agents may have played in animal deaths.

## MATERIALS AND METHODS

### Necropsy and sample collection

Steller sea lion (*Eumetopias jubatus)* fetal carcasses (n=20) were opportunistically collected by the Alaska Marine Mammal Stranding Network and Alaska Department of Fish & Game. Specimens were collected in Alaska from 1998 to 2015 and one fetal carcass was collected off the western coast of Washington (Fig. 1). Necropsies were conducted by board-certified veterinary pathologists, veterinarians, or trained biologists. Standard length (SL), curvilinear length (CL), axillary girth (AG) and weight measurements were taken following guidelines by Geraci and Lounsbury (2005). Carcass and body condition codes were assigned to all cases at the time the necropsy was conducted. Carcass classification codes include: freshly dead = 2, fair/moderate autolysis = 2.5, moderate (decomposed but organs basically intact) = 3, poor = 4 (organs not recognizable, carcass intact), mummified or skeletonized = 5 (Geraci and Lounsbury 2005). Body condition codes include robust, good, average, poor, and emaciated based on subjective indicators including prominence of bones and blubber depth. Age classifications were obtained from gross necropsy reports and included aborted fetus, intrauterine fetus, premature pup, neonatal pup and unknown. If the lungs did not float in formalin, the age was defined as an aborted fetus, if the lungs did float, the age was defined as a premature pup. Neonatal pups were identified by having fresh umbilical lumps. The intrauterine fetus was collected from a mother harvested by a subsistence hunter. Lung, placenta and skin lesion samples were collected and subsequently stored at -20 to -80 C, until analyses for histopathology, microbiology and virology were conducted. Opportunistic sampling resulted in a small number of samples collected into RNAlater (n=9). For histopathology, tissues were fixed in 10% formalin and later embedded in paraffin and sectioned at 4-5 μm at Histology Consultation Services (Eagle River, Alaska)

following previously described procedures (Hutchinson et al. 2015). Details collected from each SSL carcass included the month and year of sighting, age classification, sex, location, region, weight (kg), straight length (SL; cm), curvilinear length (CL; cm), axillary girth (cm), body condition and carcass classification (Table 1).

## Fetal Growth

Von Bertalanffy growth curves were used to estimate fetal (n=17) and neonatal weight (n=2) (kg), CL (cm) and SL (cm) as a function of month including all data with the exception of two outlying cases (von Bertalanffy 1957; Case 1,9). All data from case 1 were removed due to uncertainty in date of sighting of the carcass and lack of information on the animal. Case 19 was removed because weight was measured after tissue samples were collected at necropsy. Growth curves were estimated using the following equation:

$$L_t\ (or\ W_t) = L^{\infty}(or\ W^{\infty})[1 - e^{-K(t-t_0)}]$$

where $L_t$and $W_t$ describes the asymptotic size (CL, SL, weight) in the month the carcass was observed (t), and $t_0$ is the x-axis intercept, or the theoretical time when either length (cm) or weight (kg) is zero. K is a metabolic coefficient. The relationship between weight and length (CL and SL) was estimated by the power function equation, $W = aL^b$ (Table 2). Model parameter estimates are found in Table 2.

## Detection of *Coxiella burnetii, Brucella* spp., and *Chlamydophila*

Seventeen fetal lung tissues (n=17), two skin lesions (n=2) and a placenta (n=1) were sent to Colorado State University (CSU) for real-time PCR (qPCR) to detect the presence of C. *burnetii, Brucella* species including *B. melitensis, B.abortus, B. suis, B. ovis, B. canis,* and *B. neotomae* and all members in the genus C*hlamydophila (C.)* including *C. abortus, C. caviae, C.*

*felis, C. pecorum, C. pneumoniae* and *C. psittaci*. DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA) and real-time PCR was perfomed targeting the IS1111 and COM1 gene target region for C. *burnetii* as described by (Kersh et al. 2010) and *Brucella* spp. IS711 gene target region following methodology from Hinic et al. (2008). The qPCR amplification was performed in accordance with a previous study (Kersh et al. 2010). The gene targets and primer sequences for PCR of each pathogen are presented in Table 3. For *Brucella* detection by PCR, CSU used an additional Center for Disease Control (CDC) protocol with three target regions including BRU 1, 2 and 3. The CDC protocol defines a sample as positive if all three targets are positive and suspect if 1-2 targets are positive. Marine mammal origin B. *pinnipedialis* and C. *burnetii* positive amplification was used as a positive control with a no template negative control. A sample was considered to be positive if the cycle threshold (Ct) value was below or equal to 40 (Duncan et al. 2014). The primers used could detect all members of the *Chlamydophila* genus (Hewinson et al. 1997).

Detection of Morbilliviruses

Total RNA was extracted from subsamples of seventeen fetal lung tissues, two skin lesions and one placenta at University of Alaska (UAA) Bortz Laboratory and two skin lesions and one placenta at University of California Davis (UCD) with initial testing conducted at UCD and re-testing conducted at UAA. Tissues were frozen for various periods of time and tissues were opportunistically stored in RNAlater®. Subsamples were ground using disposable tips in a liquid nitrogen bath to break up fibrous material. Total RNA was then extracted using Invitrogen™ TRIzol™ Reagent, according to the manufacturer's instructions. RNA yield was quantified using a NanoDrop™ spectrophotometer. The two skin lesion and placenta subsamples were washed twice with 75% ethanol. RNA was obtained from all samples tested with concentrations ranging

from 54.5 (ng/µl) to 735.6 (ng/µl). Round one detection of morbillivirus viral RNA was performed using the BIO-RAD iTaq Universal SYBR Green One-Step Kit in the UAA laboratory and using a conventional PCR in the UCD laboratory. The universal morbillivirus primer sets used in round 1 detected a conserved region of the morbillivirus phosphoprotein (P) gene (Barrett et al 1993; Table 3). UAA conducted the second round using a hemi-nested PCR reaction with a Phocine Distemper Virus (PDV) assay, whereas UCD used a PDV and a Canine Distemper Virus (CDV) assay. A cDNA plasmid encoding this PDV P-gene fragment was used as a positive control (T. Goldstein, UC Davis Lab). Real-time, quantitative reverse-transcription PCR (qRT-PCR) was conducted on 100ng RNA from samples to identify any that were positive for viral RNA. Round one cDNA was amplified on a BioRad C1000 using 35 cycles, DNA denaturation occurred at 94°C for 2mins, annealing temperature was set at 56°C for 20 seconds followed by elongation at 72°C for 60 seconds. The final elongation step was at 72°C for 15 minutes with a subsequent cooling temperature of 4°C. Amplification (Ct value) and melt curve analyses were conducted in the BioRad C1000 software to analyze primer specificity. At UCD, TOPO isomerase enzyme (TOPO) activity for cloning of a PCR product for sequencing was conducted using the Invitrogen TOPO TA Cloning Kit for Sequencing with One Shot TOP10 chemically competent *E.coli*. PCR amplicons were cloned and submitted for Sanger sequencing to Eurofins Scientific in Louisville, Kentucky.

**Results**

Location of carcasses included one from Washington and all others from Alaska, including 40% from the Western SSL stock and 55% from the Eastern stock with one unknown location (Fig 1). Age classification included 35% aborted fetuses, 45% premature pups, 10% neonates and 10% intrauterine fetus (Table 1). The time of carcass collection varied from

February to July, excluding one outlying case in August. Of all cases, 45% were male and 55% were female. Weights of carcasses ranged from 4.5 kg (Intrauterine fetus (IU) fetus) to 20 kg with an average weight of 10.3 kg ± 5.2 when excluding outlying SSL case number 1 as well as SSL case number 19 due to discrepancies in weight measurements for that case. Carcass condition ranged from fresh (2) to poor (4) with 60% percent fresh (n=12), 20% mildly autolyzed (n=4), 15% in fair condition (n=3) and 5% in poor condition (n = 1). Body condition ranged from good to emaciated. Twenty percent were in good condition (n = 5), 45% (n= 9) were in average body condition, 20% (n= 4) in poor condition and 10% (n= 2) categorized as emaciated. The relationship between weight, SL and CL as a function of time was estimated using the Von Bertalanffy growth curves (Fig. 2) and a power function equation (Fig. 3) excluding the weight of SSL case number 1 and 19. Model parameter estimates are presented in Table 2. There was no apparent relationship between AG and month, and therefore these data were not included in the growth curve figures.

The significant gross necropsy findings included hemorrhage, evidence of dystocia, dermatitis and other. Typically, aborted fetuses recovered in March were haired over the dorsal midline with more complete coats over the hips and head and the ventrum was hairless (Fig. 4). Hemorrhage of varying levels and locations was present in seven animals (SSL 3,5,10,12,13,15,19; Table 7). Evidence of dystocia or entanglement in the umbilicord occurred in three (SSL 4, 6, 10; Table 7) and was characterized by regional subcutaneous edema to the head, and distinct demarcation and patterns of congestion, edema and hemorrhage (Fig. 5) suggesting the blood flow was restricted to portions of the body (Fig. 5). Seven animals had vesiculoulcerative dermatitis (SSL 8, 11, 12, 13, 14, 15,17; Table 7; Fig. 6). In one case, this extended to vesicles and ulcers on the umbilical cord (SSL 12; Fig. 6). Two animals had a

congenital defect of the cartilage and bone of the rib cage and trachea; more specifically, the trachea was distorted and tortuous with marked variations in the diameter of the lumen due to multiple nodular thickenings of the tracheal cartilages consistent with chondrodysplasia (SSL 8, 9; Table 7). In SSL case number 9, the heart was partially scavenged, however gross findings of the remainder of the heart indicated an overriding aorta with no apparent atrioventricular valves. Aorta and pulmonary arteries were of very large diameters in this animal. Emaciation was evident in two animals (SSL 12 and 20), and eight had edema in various body regions (SSL 1-2, 4-5, 10, 12, 13, 16; Table 7).

Significant histopathologic findings were minimal. Placentitis was not appreciated in any of the cases in which it was present. Skin lesions (SSL 13) were characterized by diffuse lymphocytic, neutrophilic and histiocytic dermal inflammatory infiltrate and marked dermal and subcutaneous edema and hemorrhaging. The epithelium had patches of acanthosis in which the epithelial cells appeared to be fused to a syncytium, with distinct juxta- and perinuclear vacuoles, often containing 1-3 amphophilic to basophilic inclusion bodies. In other areas, there was distinctive rounding up and separation of the subcorneal keratinocytes consistent with acanthyolytic cells (Fig. 8). Peripheral lymph nodes had a neutrophilic drainage reaction and many lymph nodes had hemorrhage with erythrophagocytosis.

The PCR results for *Chlamydophila* and *C. burnetii* were negative for all tissues. The CSU *Brucella* IS711 assay detected three positives (SSL 5, 6, 9) and one suspect positive for the IS711 marine mammal *Brucella* target sequence in lung tissues by PCR (SSL 2; Table 4 and 6). Follow-up attempts to culture *Brucella* were unsuccessful. Sequencing results for all positive and suspect cases are pending. The PCR results were repeatable. The CDC assay detected two animals as positive (SSL 6,9) and characterized two cases as suspect (SSL 2,5; Table 4). The

gross necropsy findings for the three *Brucella* spp. positives were insignificant, with one exhibiting evidence of dystocia and bronchopneumonia that was not present in any of the other animals. The suspect *Brucella* spp. positive cases may be explained by environmental contamination, poor sample quality, low genome copy numbers, or may represent false positives.

Two skin lesion tissues were positive by PCR for PDV (SSL 11, 13) at UCD and bone marrow and one lung tissue sample was considered suspect positive at UAA (SSL 12, 20; Table 6). The lung tissues of all animals were negative for morbilliviruses. Two skin lesions (SSL 11, 13), bone marrow (SSL 13) one placenta (SSL 13; Table 6) were positive for PDV by PCR. PCR results for two skin lesion samples (SSL11, 13) and placenta (SSL 13) were confirmed by sequencing, whereas, the bone marrow sample was only positive by PCR and negative when sequenced, suggesting the sample may have been a false positive (SSL 13; Table 6). The two animals positive for PDV had vesiculoulcerative dermatitis (SSL 11, 13; Fig. 6a), myocardial hemorrhage and edema (Fig. 6a) with possible hepatitis (SSL 13; Table 7). The skin lesion sample (SSL 12) that was suspect positive at UAA had ulcerative dermatitis, similar to the lesion seen in the skin lesion samples from SSL case number 11 and 13. The other suspect positive, SSL case number 20 had no gross pathology signs suggestive of disease. Case 12 and 20 were both emaciated.

**DISCUSSION**

Infectious disease agents *Coxiella burnetii, Brucella* spp, *Chlamydophila* and morbilliviruses can cause reproductive failure and neonatal mortality that has the potential to affect population trajectory (Gaydos et al. 2004; Borel et al. 2006). The present study expands upon previous research efforts by Burek et al. (2005) in determining if *Coxiella burnetii, Chlamydophila, Brucella* spp. and morbilliviruses contribute to SSL reproductive failure. PCR

was used for pathogen detection to determine infection rather than conducting additional serology studies that are limited to identifying prior exposure to a pathogen or related pathogen. The majority of our results were negative with exception of *Brucella* spp. and morbillivirus. Although SSL infectious disease studies are limited, other studies have detected *Brucella* spp. by PCR in northern fur seals (*Callorhinus ursinus;* Duncan et al. 2014*)* and harbor seals (Hoover-Miller et al. 2017), and isolated zoonotic *Brucella* strain ST27 in California sea lions (Goldstein et al. 2009), suggesting the ability of this pathogen to infect sympatric species.

Data compiled in Table 1 provide valuable insight into time, sex, location and morphometrics of aborted and premature fetuses and the neonatal deaths. The time frame in which the fetal and neonatal carcasses were collected range from February to July. We suspect the August SSL from Washington was not collected in August given that it only weighed 4 kg and an abortion is highly unlikely to occur this late in the year. With exception to the neonatal SSL case number 19 and 1, all fetal carcasses were collected between February and May, which is expected, as pregnancy rates are typically lower during that time period (Pitcher and Calkins 1981). The weight and length data fit the von Bertalanffy growth curves reasonably well, approaching an asymptote in May (Fig. 2). The relationship between fetal length and weight was also estimated by the power function equation in which weight increased as SL and CL increased by a factor of b (Fig. 3). Although we were unable to compare our growth curves to other SSL fetal growth curves, we were able to compare the weight to other research findings. The neonatal pups 16 and 20 were included in the present study because they were not suspected to have lived longer than 1-5 days, and were fairly close in weight, SL, CL and AG to other cases. Additionally, diseases that cause late term abortion are often associated with weak neonatal animals. A study conducted in Southeast Alaska, the Gulf of Alaska, and Aleutian Islands found

the average weight of neonatal pups at 1-5 days old to be 22.4 ± 2.36 kg for males and 18.7 ± 2.08 kg for females, which is higher than the weight of the two neonates in the present study (Brandon et al. 2004). This is expected considering the body condition of the neonates in this study was poor (SSL 16) and emaciated (SSL 20; Table 1) and likely contributed to mortality. The male premature pup found in May weighed 15.4 kg, whereas the premature female pup and male aborted fetus found in March and April weighed 20 kg, which seems rather high if comparing it to the average neonatal pup weight illustrated by Brandon et al. 2004 in May and June. Further work should be conducted to determine SSL fetal growth similar to that done for other otariid species.

The present study suggests C. *burnetii* and *Chlamydophila* did not cause spontaneous abortion, neonatal and/or premature SSL death in any of the cases (n=20). Kersh et al. (2010) and Lapointe et al. (1999) detected C. *burnetii* in placental tissues (n=1), but for the majority of cases (n=19), the placentae were unavailable for testing. Following infection, if the bacteria penetrate the placenta, it is possible to infect the fetus via the amniotic-oral route (Agerholm 2013) thus testing of lung tissues was performed Bradley et al. (1994) successfully isolated a chlamydial organism, identified at the time as *Chlamydia psittaci* from a SSL aborted fetal lung using cell culture but was unable to further characterize the isolate. Despite antibody presence to Cp. *psittaci* in SSL (Burek et al. 2005), no evidence of actual detection of Cp. *psittaci* in SSL using molecular techniques has been reported. Unpublished data on forty SSLs from different regions of Alaska did detect *C. psittaci* from oral, rectal and vaginal swabs by PCR at approximately 50% using a commercial lab primer for *C. psittaci* (Kathy Burek-Huntington, Alaska Veterinary Pathology Services, personal communication September 1, 2015).

Three cases were positive for *Brucella* spp., and one was suspect. The suspect positive had a Ct value of 44 on the first run and 37 on the second repeat run, suggesting the tissue sample contained low levels of DNA, was a false positive, contaminated, or cross-reactivity occurred (Table 4; Case 2). The positive findings for SSL case number 5, 6 and 9 are novel, considering this is the only confirmed detection of marine *Brucella* by PCR in a SSL. The only positive SSL case previously found was in an adult female (Burek et al. 2005), using serological techniques. The PCR assays used in the present study were validated (Hinić et al. 2008). Thus far, detection of *Brucella spp.* in otariid seals inhabiting Alaska waters has only been in northern fur seals (*Callorhinus ursinus)* and one case exhibited severe placentitis (Duncan et al. 2014). Considering the detection of zoonotic *Brucella* strain ST27 in California sea lion placentae (Goldstein et al. 2009), and associated pathology, there is reasonable concern on the affect *Brucella spp.* may have on eared seals in Alaskan waters given its ability to cause morbidity and possible reproductive failure in other species. The three positive SSL cases were in average (SSL 5, 9) and good (SSL 6) body condition. Inflammation was not present in the lungs in the PCR positive animals. Similar to Godfroid (2002) findings, the positive tissues in the present study had no clear signs of associated infection. High Ct values from the positive lung tissues indicate low levels of bacterial DNA, suggesting *Brucella* likely did not contribute to abortion (Table 4). However, it is important to recognize the placentae were not available for examination, and thus the role of *Brucella* in abortion remains unknown.

Phocine Distemper Virus (PDV) was detected in three of the four aborted pups (SSL11,12, 13; Table 6) from the Kodiak "abortion storm," with two of those confirmed positive and one suspect positive based on PCR (Fig. 9). It is also possible that PDV may have been positive in a neonatal death (SSL 20; Table 6). This study provides rather novel information on

the detection of PDV in the placenta and skin of SSL by PCR which may be associated with pathology as suggested by the inclusion body present in the flipper of one case (SSL 13). Gross necropsy findings included vesiculoulcerative dermatitis and stomatitis from the skin lesion (SSL 12, 13; Table 7) with inclusion bodies in one case. PDV has been associated with skin lesions in marine mammals previously and morbilliviral dermatitis was first documented in a hooded seal (*Cystophora cristata)* (Lipscomb et al. 2001). Although abortion and stillbirth occurred in the 1988 and 2002 harbor seal mass mortalities, there was insufficient evidence to support PDV as the causative agent and it was presumed to be secondary to illness and death in the dam (Duignan et al. 2014). The present study suggests vertical transmission of PDV may have occurred in SSL. Currently, the role of PDV infection in the North Pacific is unknown, as 41% of northern sea otters in Kodiak tested seropositive for antibodies to PDV (Goldstein et al. 2009). Goldstein et al. (2011) also detected a PDV fragment identical to the isolate from the 2002 European harbour seal outbreak in eight live sea otters and three dead sea otters along eastern Aleutian Islands and Kodiak Archipelago using a hemi-nested PCR. Thus, an understanding of the presence of PDV infection in sympatric species in the North Pacific is needed to better understand the risk to SSL. Given the lack of information on PDV infections in the SSL continued monitoring is also needed.

Other interesting findings include the presence of congenital defects in 2006 in Southeast Alaska (SSL 8, 9; Table 7) and were likely the cause of abortion. This is of particular interest as congenital disorders and genetic defects can also cause reproductive deaths in marine mammals (Geraci and Lounsbury 2009; Murphy et al. 2013; Reeves et al. 2001; Geraci et al. 1999). SSL case 8 had severe congenital defects of the skeletal system, more specifically, abnormal development of the cartilage suggestive of a chondrodysplasia. This pup also had vesicular

dermatitis, which is similar pathology to that observed in the positive PDV skin SSL cases 11 and 13 (Table 7); however, there were no positive lab results to support this. Furthermore, the characteristics of the observed pathology are unlike the limited findings of morbiliviral dermatitis in pinnipeds. SSL case 9 had valvular dysplasia of the left and right atrioventricular valves. Gross necropsy findings indicated the heart defect was the cause of death for this case.

We attempted to understand if *C. burnetii, Brucella* spp., *Chlamydophila* and PDV play a role in spontaneous abortion, premature pup and neonatal deaths in SSL. Although our findings were unable to detect active infection with *C. burnetii* and *Chlamydophila*, they did provide the first molecular detection of marine *Brucella* in a SSL and gave novel insight into the pathology of PDV in SSL. We fully recognize the sample size and geographic range covered in this study is insufficient to make conclusions about the ability these pathogens to affect the SSL population numbers due to reproductive failure. Opportunistic sampling and additional lab analyses conducted on other SSL aborted and premature pups were excluded due to inconsistencies in disease diagnostic tools utilized in various labs. In conclusion, given that PDV was detected, it may have played a contributory role to the pup deaths on Kodiak, AK. It is unclear whether *Brucella* spp. contributed to reproductive failure, however it seems unlikely given the absence of histologic lesions.

Disease is only one contributing factor to spontaneous abortion and premature parturition in SSL. Contaminants and harmful algal bloom exposure may also play a role (DeLong et al. 1973; Brodie et al. 2006), both of which go beyond the scope of the present study. It is also important to recognize abortion as a potential reproductive strategy of otariids during periods of nutritional stress in order to increase their reproductive performance (Testa 1987; Pitcher et al. 1998). However, it should also be recognized that pinnipeds evolved to practice embryonic

diapause which would serve the same purpose acting earlier in gestation with less energetic costs. Breeding in otariid species has been described as "energetically expensive" (Costa 1993), and as a result SSL may have evolved multiple lactation and gestational mating strategies to allow for optimal reproductive success (Atkinson 1997). Despite our small sample size, this study only found two cases that were emaciated, (Case 12, 20; Table 7) with no other findings suggestive of nutritional stress. Of the two emaciated cases, SSL case 12 had ulcerative dermatitis with no other significant gross necropsy findings. Through our analyses and review of other studies, we have determined there is still great uncertainty how these pathogens contribute to SSL reproductive health. This research encourages further studies to include molecular analyses, serological surveys in conjunction with pathology to better understand the role infection may play.

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Kristy Pabilonia with Colorado State University and Tracey Goldstein with University of California Davis for part of tissue analyses and assistance with obtaining proper protocols; Rasmuson Research Fisheries Center provided support for this project.

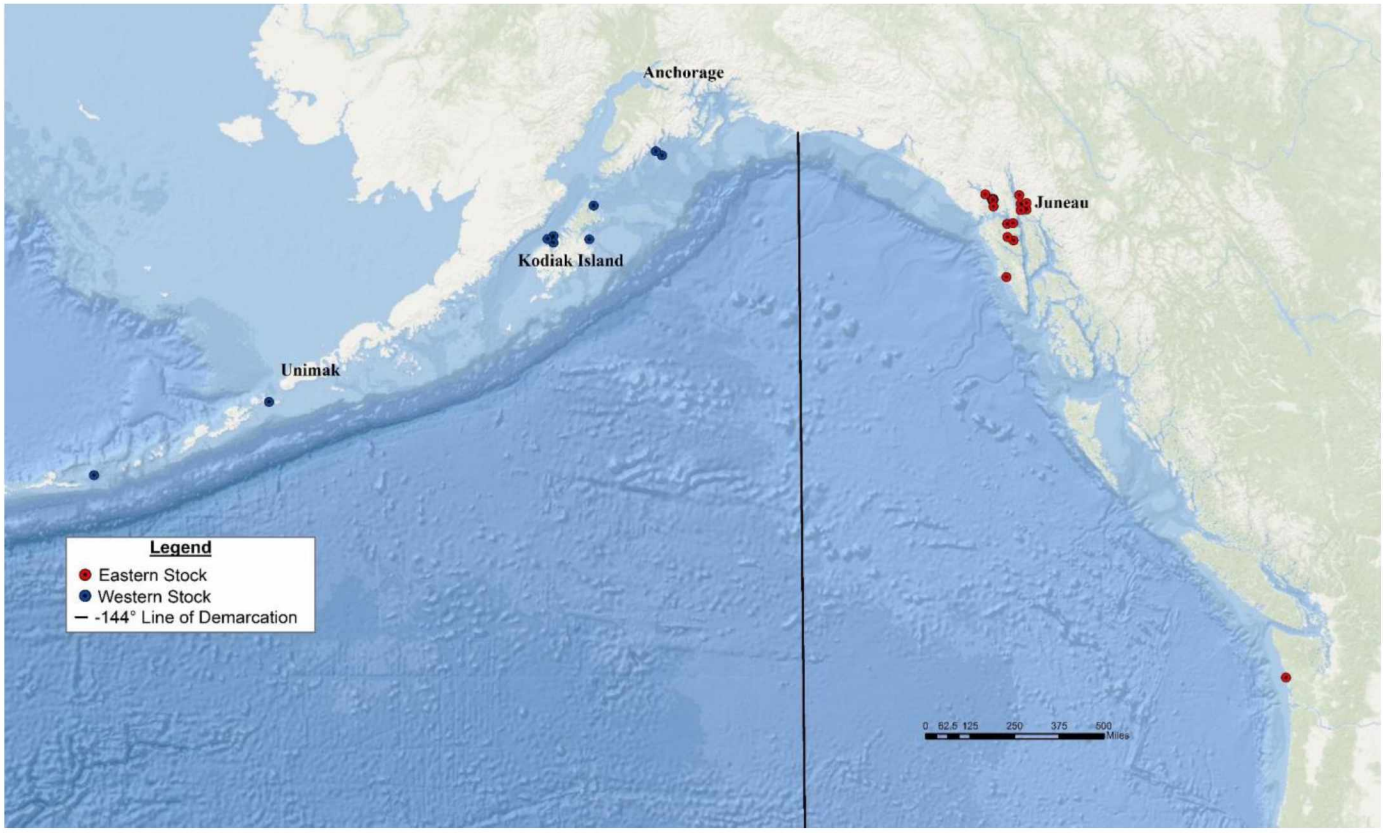


Fig. 1. Study area map illustrating location of Steller sea lion aborted and intrauterine fetuses, premature and neonatal deaths. The blue color markers indicate deaths occurring West of Cape Suckling, 144° West longitude, and the red color markers indicate deaths occurring east of that point. The study area includes death occurrences as far west as Basalt Rock in the eastern Eastern Aleutian Islands, to as far south as Ocean Shores, Washington.

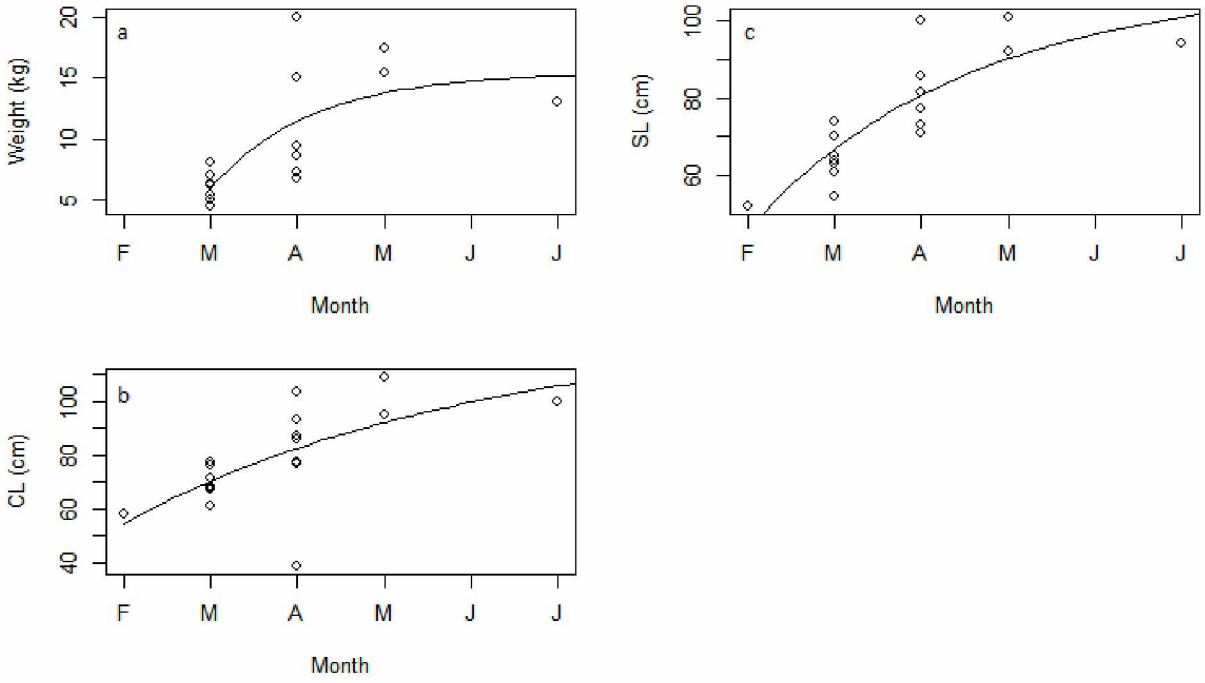


Fig. 2. Von Bertalanffy growth curves for SSL fetal and neonatal cases a) weight, b) curvilinear length (CL), and c) straight length (SL) as a function of month. Weights are measured in kg and lengths are measured in cm.

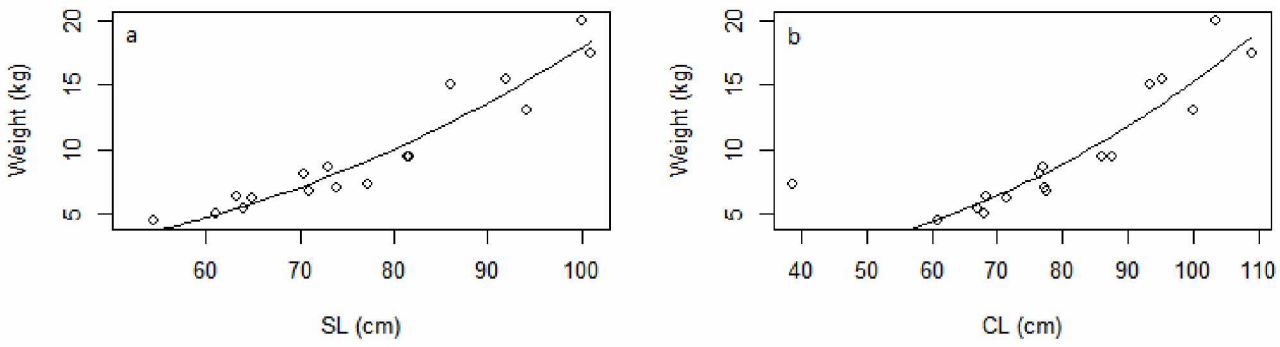


Fig. 3. SSL fetal and neonatal weight as a function of a) straight length (SL) and b) curvilinear length (CL). Weights are measured in kg and lengths are measured in cm

Fig. 4. Ventral view with minimal hair growth in SSL 12. Dorsal surface is fully haired (not shown above).

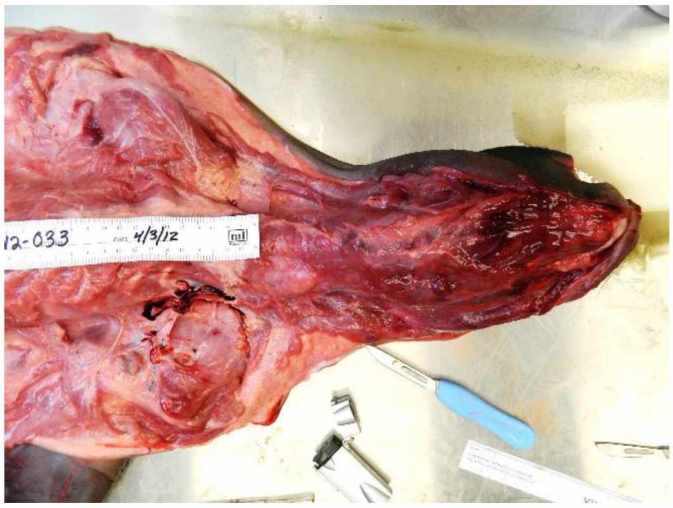


Fig. 5. Differential congestion suggesting entrapment in the birth canal or strangulation by the umbilical cord in SSL 12.


Fig. 6a. SSL 12 exhibiting vesiculoulcerative dermatitis with lesions particularly evident on the ventrum and flippers.

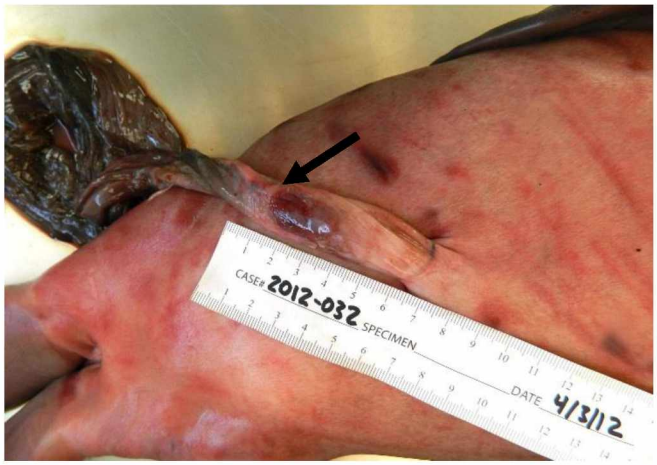


Fig. 6b. SSL 12 with large vesicles on the umbilical cord (arrow).

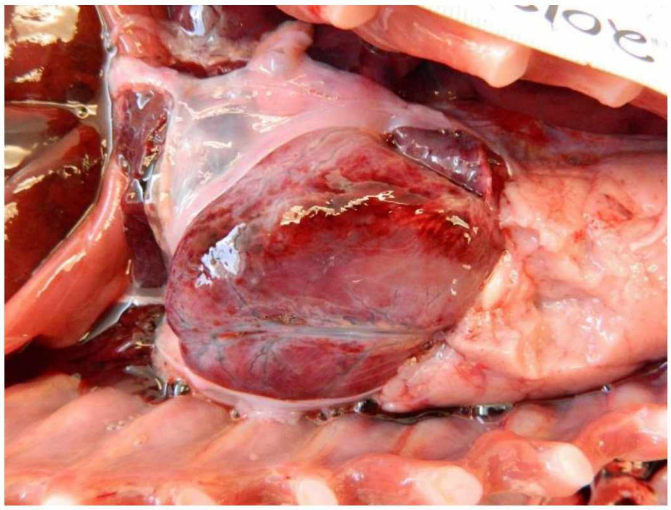

Fig. 7. SSL 12 with epi- and endocardial petechia and ecchymoses.

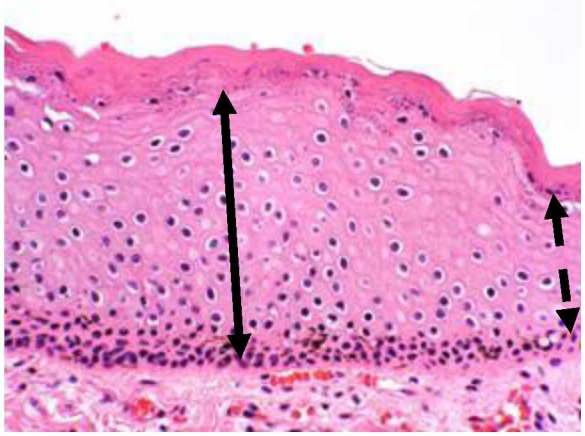

Fig. 8a. Hind flipper from SSL 13 showing a diffuse lymphocytic, neutrophilic and histiocytic dermal inflammatory infiltrate. The dermis and subcutis are markedly edematous and hemorrhagic. The epithelium has patches of thickened epithelium due to increased thickness of the stratum spinosum (solid arrow) vs. the more normal thickness (dotted arrow) (40x).

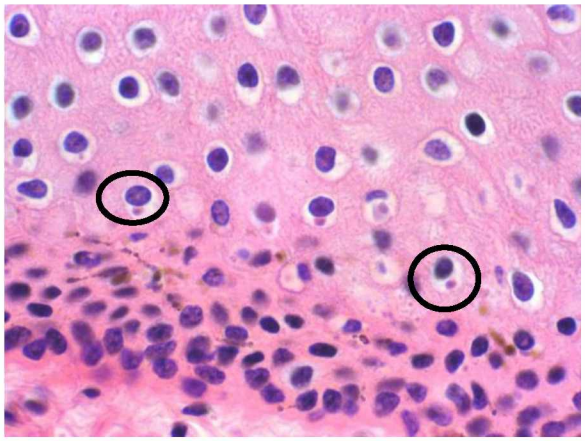

Fig. 8b. The epithelial cells of SSL 13 flipper often look fused into a syncytium, with distinct juxta- and perinuclear vacuoles, often containing 1-3 amphophilic to basophilic inclusion bodies (circles) 100x.

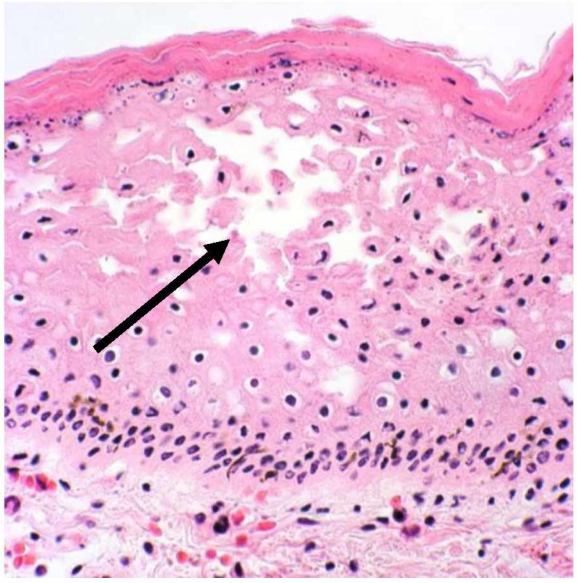

Fig. 8c. SSL 13, showing other areas where there is distinctive rounding up and separation of the subcorneal keratinocytes consistent with acanthocytes (arrow) 40x.

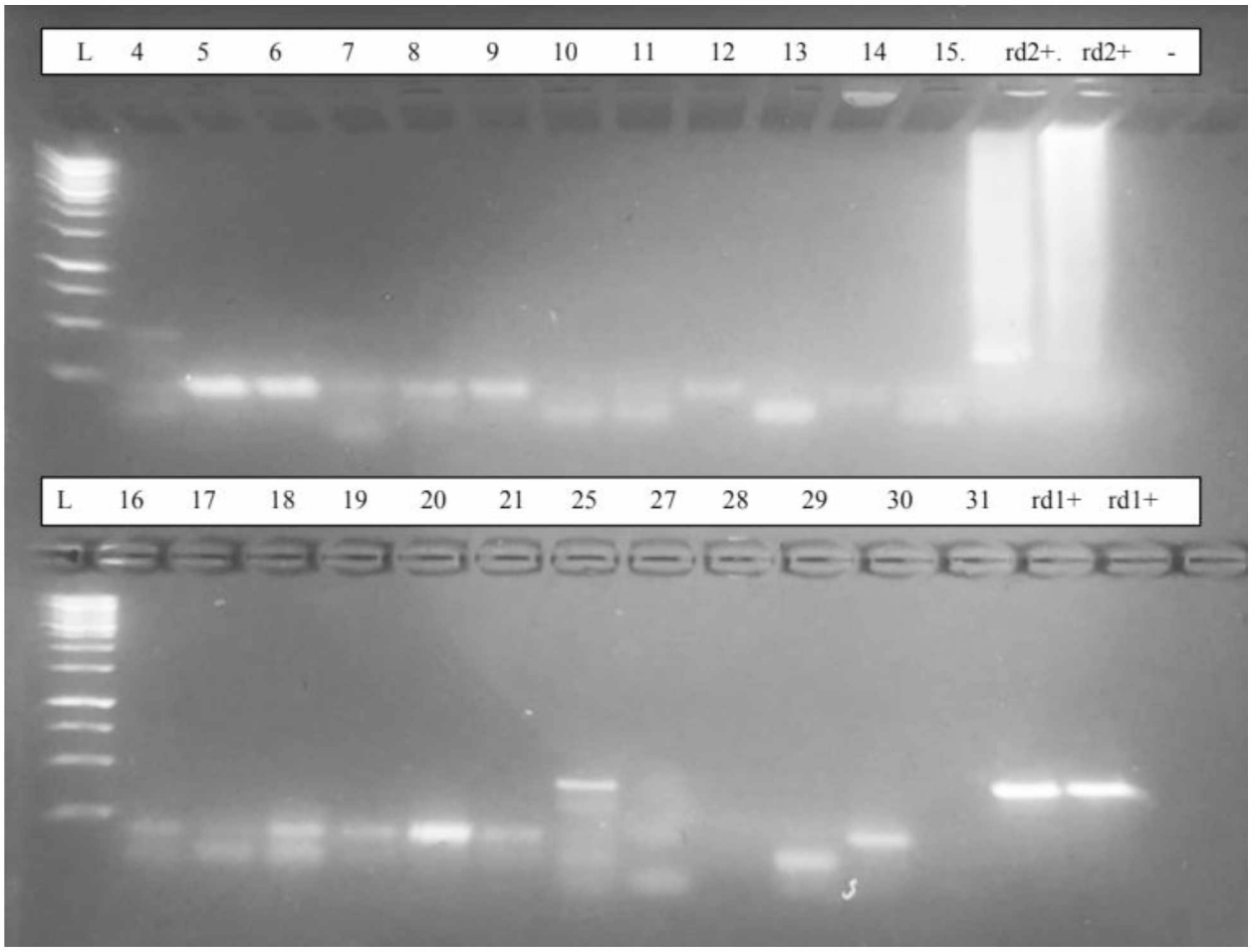


Fig. 9. Gel image of products from the conventional PCR from round II heminested PDV assay at UAA. SSL numbers do not align with well numbers. Lane 4 (SSL 20) and lane 25 (SSL 12) exhibit amplifications for PDV, near 500BP. All size estimates were based on the ThermoScientific 1 kb gene ruler.

Table 1. Steller sea lion (*Eumetopias jubatus*) aborted fetuses, premature pups, neonatal death and intrauterine fetus from harvested or stranded adult female (IU) cases identified by month (Mo.) and year (Yr.), age class, sex (F: female; M: male; Unknown: UNK), location, region, weight (Wt), straight length (SL), curvilinear length (CL), axillary girth, body condition and carcass classification.

| SSL Num. | Mo. | Yr. | Age Classification | Sex | Location | Region | Wt (kg) | SL (cm) | CL (cm) | Axillary Girth (cm) | Body Condition | Carcass Classification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | Aug | 1998 | Aborted | F | Ocean Shores | WA | 4 | 58 | 64.5 | 33.8 | Average | 2.5 |
| **2** | Mar | 2002 | Premature | F | Basalt Rock | Eastern Aleutian | 5 | 61 | 68 | 38 | Good | 2 |
| **3** | Mar | 2004 | Aborted | M | Benjamin Island | Southeast | 7 | 74 | 77.3 | 40.9 | Average | 2 |
| **4** | Apr | 2004 | Aborted | M | Berners Bay | Southeast | 20 | 100 | 103.5 | 56.5 | Poor | 2 |
| **5** | Mar | 2004 | Premature | F | Benjamin Island | Southeast | 20 | 71.6 | 81.6 | 44 | Average | 3 |
| **6** | Mar | 2005 | Premature | M | Benjamin Island | Southeast | 6.3 | 65 | 71.5 | 41.5 | Good | 2.5 |
| **7** | Mar | 2005 | IU Fetus | F | UNK | UNK | 8.1 | 70.4 | 76.4 | 43.4 | Average | 2 |
| **8** | Mar | 2006 | Aborted | F | Benjamin Island | Southeast | 5.5 | 64 | 67 | 45 | Good | 2 |
| **9** | Apr | 2006 | Aborted | F | S. Marble Island | Southeast | 9.5 | 81.6 | 86 | 64 | Average | 2.5 |
| **10** | Apr | 2008 | Premature | M | Gran Rock | Southeast | 15 | 86 | 93.5 | 54.5 | Good | 2 |
| **11** | Apr | 2012 | Premature | M | Cape Ugat | Kodiak | 7.3 | 77.2 | 38.7 | 38.7 | Poor | 4 |
| **12** | Apr | 2012 | Aborted | M | Cape Ugat | Kodiak / GOA | 9.5 | 81.5 | 87.5 | UNK | Emaciated | 3 |
| **13** | Apr | 2012 | Premature | F | Cape Ugat | Kodiak / GOA | 6.8 | 71 | 77.5 | 39.9 | Average | 2 |
| **14** | Mar | 2013 | Premature | F | Sea Otter Island | Kodiak / GOA | 6.4 | 63.2 | 68.4 | 40.6 | Average | 2 |
| **15** | Mar | 2013 | IU Fetus | F | Sitka | Southeast | 4.5 | 54.5 | 61 | 38 | Good | 2 |
| **16** | May | 2013 | Neonate | M | Chiswell Islands | Gulf of Alaska | 17.4 | 101 | 109 | 56.5 | Poor | 2 |
| **17** | May | 2013 | Premature | M | Kodiak Harbor | Gulf of Alaska | 15.4 | 92 | 95.2 | UNK | Poor | 2 |
| **18** | Apr | 2014 | Aborted | M | Tenakee | Southeast | 8.6 | 73 | 77 | 36.5 | Average | 2 |
| **19** | Feb | 2015 | Premature | F | Resurrection Bay | South central | UNK | 52 | 58 | 29.7 | Average | 3 |
| **20** | Jul | 2015 | Neonate | F | Tenakee | Southeast | 13 | 94.2 | 100 | 46.3 | Emaciated | 2.5 |

Table 2. Growth model equation and corresponding parameters estimates (± SE) for $L^{\infty}$, $W^{\infty}$, $t_0$ and K representing the length [curvilinear length (CL); standard length (SL)] and weight of fetal and neonatal carcasses over time, t and the power function equation parameter estimates for a and b, in which weight is a function of length.

| **Model** | **Equation** | **Length (cm)** | | |
|---|---|---|---|---|
| **von Bertlanaffy** | $L_t = L^{\infty}[1 - e^{-K(t-t_0)}]$ | $\boldsymbol{L^{\infty}}$ | $\boldsymbol{t_0}$ | **K** |
| | CL (cm) | 127 ± 70.1 | -0.31 ± 0.38 | 0.24 ± 2.55 |
| | SL (cm) | 110 ± 18.3 | 0.39 ± 0.22 | 0.61 ± 0.75 |
| | $W_t = W^{\infty}[1 - e^{-K(t-t_0)}]$ | $\boldsymbol{W^{\infty}}$ | $\boldsymbol{t_0}$ | **K** |
| | | 15.5 ± 3.96 | 2.43 ± 0.41 | 0.86 ± 0.75 |
| **Power Function** | $W = aL^b$ | **a** | **b** | |
| | CL (cm) | 0 | 2.43 ± 0.32 | |
| | SL (cm) | 0 | 2.59 ± 0.22 | |

Table 3. Target pathogen, gene target sequence, primer sequences, probe sequences and corresponding references for PCR and two hemi-nested PCR reactions including a PDV and CDV assay used in this study.

| Target Pathogen | Target sequence | Forward primer/reverse primer (5' → 3') | Probe (5' Fluorophore → 3' Quencher) | Reference |
|---|---|---|---|---|
| ***Brucella*** | IS711 | GCTTGAAGCTTGCGGACAGT/GGCCTA CCGCTGCGAAT | FAM-AAGCCAACACCCGGCCATTATGGT-TAMRA | Hinic et al. 2008 |
| ***Coxiella burnetii*** | COM1 | AATAAAAACCTCCGCGTTGTCTT/TTGG CAGCGTATTGCGATT | AAAGAACTGCCCATTTTTGGCGGC | Kersh et al. 2010 |
| ***Coxiella burnetii*** | IS1111A | CCGATCATTTGGGCGCT/CGGCGGTGTT TAGGC | TTAACACGCCAAGAAACGTATCGCTGT G | Kersh et al. 2010 |
| ***Chlamydophila*** | NA | GCATAATCTTTAGAGGTGAGTATGA/C CTTCCCACATAGTGCCATCG | NA | Hewinson et al. 1997 |
| **Morbillivirus** | NA | ATGTTTATGATCACAGCGGT/ ATTGGGTTGCACCACTTGTC | NA | Barrett et al. 1993 |
| **Morbillivirus/ Phocine Distemper Virus** | NA | ATGTTTATGATCACAGCGGT/GTTCGCA TGCTTGGTTCCCTATAC | NA | Goldstein et al. 2011 |
| **Morbillivirus/ Canine Distemper Virus** | NA | ATGTTTATGATCACAGCGGT/TAATGGA TCCACACTCCGGATCC | NA | Goldstein et al. 2011 |

Table 4. Steller sea lion case number, positive and suspect positives for marine mammal *Brucella* by the IS711 CSU assay and CDC assay and associated Ct values for the initial, repeat and 2nd repeat PCR run.

| SSL Number | Initial IS711 Ct | Repeat IS711 Ct | 2nd Repeat IS711 Ct | LRN BRU1 Ct | LRN BRU2 Ct | LRN BRU3 Ct | Interpretation |
|---|---|---|---|---|---|---|---|
| 2 | 44.32 | 0 | 37.06 | 0 | 35.69 | 0 | Suspect positive on both assays. |
| 5 | 37.90 | 40.82 | 36.44 | 39.43 | 35.26 | 0 | Positive on IS711 assay and suspect positive on CDC assay |
| 6 | 37.18 | 41.43 | 35.29 | 37.12 | 32.63 | 37.43 | Positive on IS711 assay and positive on CDC assay |
| 9 | 28.57 | 31.29 | 27.17 | 31.00 | 25.69 | 31.09 | Positive on IS711 assay and positive on CDC assay |

Table 5. Steller sea lion case number, specimen type and PCR results for *Brucella* spp., *C. burnetti.* and *Chlamydophila.*

| SSL Number | Specimen Type | *Brucella* spp. | *C. burnetti* | *Chlamydophila* |
|---|---|---|---|---|
| **1** | Lung Tissue | NEG | NEG | NEG |
| **2** | Lung Tissue | SUS | NEG | NEG |
| **3** | Lung Tissue | NEG | NEG | NEG |
| **4** | Lung Tissue | NEG | NEG | NEG |
| **5** | Lung Tissue | POS | NEG | NEG |
| **6** | Lung Tissue | POS | NEG | NEG |
| **7** | Lung Tissue | NEG | NEG | NEG |
| **8** | Lung Tissue | NEG | NEG | NEG |
| **9** | Lung Tissue | POS | NEG | NEG |
| **10** | Lung Tissue | NEG | NEG | NEG |
| **11** | Lung Tissue | NEG | NEG | NEG |
| **12** | Skin Lesion | NEG | NEG | NEG |
| **13** | Lung Tissue | NEG | NEG | NEG |
| **14** | Lung Tissue | NEG | NEG | NEG |
| **15** | Placenta | NEG | NEG | NEG |
| **16** | Lung Tissue | NEG | NEG | NEG |
| **17** | Skin Lesion | NEG | NEG | NEG |
| **18** | Lung Tissue | NEG | NEG | NEG |
| **19** | Lung Tissue | NEG | NEG | NEG |
| **20** | Lung Tissue | NEG | NEG | NEG |

Table 6. Steller sea lion case number, specimen type, University of Alaska Anchorage Bortz Lab* and University of California Davis PCR and sequencing results for PDV. I=No amplifiable RNA in sample. **denotes cases that were positive for PDV by PCR and further sequencing in the Bortz Lab.

| SSL Number | Specimen Type | PDV | Sequencing** |
|---|---|---|---|
| **1** | Lung* | NEG* | |
| **2** | Lung* | NEG* | |
| **3** | Lung*<br>Lung | NEG*, NEG | |
| **4** | Lung* | NEG* | |
| **5** | Lung* | NEG* | |
| **6** | Lung*, Brain, Lung, Lymph Node | NEG*, NEG, I, I | |
| **7** | Lung* | NEG* | |
| **8** | Lung*, Nasal Swab, | NEG*, NEG | |
| **9** | Lung* | SUS* | |
| **10** | Lung*, Brain, Lung, Lymph Node | NEG*, NEG, NEG, I | |
| **11** | Lung*, Skin Lesion, Thymus, Brain, Lymph Node Prescapular | NEG*, POS, NEG, NEG, I | Skin lesion confirmed positive |
| **12** | Lung*, Skin Lesion*, Conjunctiva, Brain and Rectal Dry Swabs, Tissue Pool, Liver, Lymph Node axillary, Lymph Node hilar, Stomach Contents, Thymus | NEG*, SUSP*, NEG for remaining tissues and swabs | |
| **13** | Lung*,<br>Skin Lesion, Placenta, Bone Marrow, Brain, Conjunctiva, Nasal, Oral, Rectal, Spleen Dry Swabs, Lung, Thymus | SUSP*, POS, POS, POS, NEG for remaining tissues and swabs | Skin lesion and placenta confirmed positive |
| **14** | Lung*, Lymph Node Prescapular, Lung, Nasal Dry Swab, Skin, Thymus | NEG*, NEG for remaining | |
| **15** | Placenta*, Conjunctiva Dry Swab, Placenta, Skin Lesion | NEG*, NEG for remaining tissues and swabs | |
| **16** | Lung Tissue* | NEG* | |
| **17** | Skin Lesion*, Tissue Pool, Skin lesion | NEG*, NEG | |
| **18** | Lung Tissue* | NEG* | |
| **19** | Lung Tissue* | NEG* | |
| **20** | Lung Tissue* | PROB POS* | |

Table 7. Steller sea lion case number and significant gross necropsy findings including hemorrhage, dystocia, dermatitis and other notes documenting additional findings. SSL case numbers positive for disease agents denoted by POS.

| SSL Number | Hemorrhage | Evidence of Dystocia | Dermatitis | Disease agents | Notes |
|---|---|---|---|---|---|
| 1 | N | N | N | | Trauma with laceration of the liver and hemoperitoneum; Mild pulmonary edema |
| 2 | N | N | N | Suspect Brucella POS - Lung | acute hemorrhage in the epicardium, vacuolar nephropathy |
| 3 | Y epi- and endocardium | N | N | | Generalized organ congestion; pigmentary hepatopathy, pneumonia, focal, pyogranulomatous, associated w/hair shaft, drainage of acute hemorrhage cervical lymph node; Degnerative myopathy |
| 4 | N | Y | N | Brucella POS - Lung | Regional SQ edema to the head and pulmonary edema; trauma with liver fracture |
| 5 | N | N | N | Brucella POS - Lung | Trauma with SQ hemorrhages and edema involving neck, head chest and abdomen. |
| 6 | N | Y | N | | Antemortem trauma to head, fractured liver and puncture wounds to body. Generalized organ congestion; muscle degeneration; epicarditis, mixed, pulmonary edema, hemorrhage, congestion etc. |
| 7 | N | N | N | | Normal fetus collected during subsistence hunt. Minor focal trauma to head and thorax. Extracted from mother. |
| 8 | N | N | Y, vesicular dematitis | | Severe condrodysplasia (congenital defect) |
| 9 | N | N | N | Brucella POS - Lung | Cardiac congenital defect |
| 10 | N | Y | N | | Congestion, edema and hemorrhage |
| 11 | N | N | Y, ulcerative dermatitis | PDV POS - Skin lesion | Ulcerative dermatitis; mild suppurative lymphadentitis of peripheral lymph nodes; cardiac hemorrhage, poor body condition |
| 12 | Y endocardium | Y | Y, ulcerative dermatitis | PDV PROB POS- Skin lesion | Several shallow erosions/ruptured vesicles/ulcers around the ventral pelvic area and umbilicus. |

| | | | | | |
|---|---|---|---|---|---|
| 13 | N | N | Y, vesicular and ulcerative dermatitis | PDV POS-Skin lesion and placenta | Myocardial hemorrhage and edema. Stomatitis. Scattered ruptured vesicles on its abdomen, neck, and hindflippers. |
| 14 | N | N | Y- vesicular dermatitis | | Meconium staining indicated fetus stress before expulsion; vesicular lesions on the HFs; Mild lymphocytic epicarditis and endocarditis. |
| 15 | N | N | Y, possible vesicular skin disease | | Intrauterine fetus of mother that died of unknown causes. Acute hemorrhage suggesting trauma to the mother. |
| 16 | N | N | Spongiosis, focal with ulcerative dermatitis and intralesional bacterial cocci. | | Suspect death due to inanition. Mild pulmonary congestion and edema. focal w/ulcerative dermatitis with spongiosis and bacteria |
| 17 | N | N | Y, on skin, right axillary; lymphoplasmacytic; hydropic degeneration | | Minor trauma. mild erosions in the mouth and possible vesicular skin lesions on the umbilicus, |
| 18 | N | N | N | | Head trauma |
| 19 | N | N | N | | Subcutaneous hemorrhage on back of head and right shoulder. |
| 20 | N | N | N | PDV PROB POS- Lung | Emaciated and likely asphyxiated. Possible an in-utero infection occurred or poor nutritional state of the mother; Omphalitis |

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## General Conclusions

*Strandings Trends*

SSL were selected as the species of focus in my thesis to address the potential role anthropogenic and disease factors may play on SSL strandings and more specifically, on reproductive health. These factors in addition to malnutrition, predation, and climate change are suspected to have contributed to the well-known SSL decline (Loughlin 1998, Trites and Donnelly 2003, Burek et al. 2005, Atkinson et al. 2008). Researchers continue to investigate factors contributing to the dichotomous trajectories of the western and eastern SSL stock, which has led to the endangered listing of the western SSL stock and the delisted eastern SSL stock. Chapter 1 of my thesis utilized SSL stranding data (n=1507) from 1990-2015 collected in Alaska, Washington and Oregon. This is the first utilization and analyses of stranding data collected from Alaska. The main objectives of Chapter 1 of this study were to examine trends in SSL strandings, detect geographic areas of high stranding occurrences, identify annual and seasonality trends for both stocks and to identify signs of human interaction of strandings. There are several caveats associated with the data due to opportunistic data collection and inconsistent information available from the Alaska Region and West Coast (WA, OR) Region Stranding Network. However, we did quantify effort by analyzing differences in grant funding and stranding stakeholders between the regions.

We determined adult males as the most frequently occurring age class to strand in both regions, which is supported by findings of Shuert et al. (2015) who found the mean survival rate for male SSL to be lower than that of females. Males also have more extensive and variable movements (Raum-Suryan et al. 2002), which may help to explain the higher frequency of male strandings. The mean number of strandings across all seasons in the Alaska Region was $1.8 \pm 2.3$

with clear seasonal patterns and the highest stranding occurrences documented in the summer for the Alaska Region and in both spring and summer in the West Coast Region. This is consistent with findings from Lee (2016), where strandings of SSL in Southern Washington and Oregon are highest in the summer. Additional GLM analyses indicated the proportion of strandings in the spring and summer to be significantly higher in the Alaska Region versus the West Coast Region suggesting region may account for the observed seasonality variation ($p < 0.05$). We recognize the vast differences in coastal human populations in the Alaska Region and West Coast Region may contribute to the differences in observed seasonality variation. Other findings indicate the regions with the highest number of stranding reports in Alaska to correspond with the highest human coastal populated areas in Alaska, illuminating the caution one must take when attempting to correlate stranding frequency with SSL population trends. The last component of Chapter 1 characterized signs of human interaction.

We were unable to completely determine the anthropogenic factors that could have played a role in SSL strandings due to the inability to determine signs of human interaction for 50% of cases in the Alaska Region and 70% of cases in the West Coast Region. The available data on human interaction cases indicate a large proportion of human interaction cases (i.e. 81%) to occur between 2008-2015 in both regions. Factors of human interaction included gun-shot wounds and boat collision, suggesting these deaths were likely a result of a fishery-related interaction. 40% of the cases identified as shot in Alaska occurred in 2015, in which seven of the cases were involved in a Copper River commercial salmon drift gillnet fishery. Therefore, we must continue to evaluate the effects of fishery-related interactions on SSL populations.

In Chapter 1, we attempted to identify SSL stranding trends over a 25-year period in three different states. Our findings indicate trends in seasonality and sex are fairly consistent with life

history strategies possessed by SSL. However, the high level of unknown sex, age class and human interaction cases prevented us from determining cause-specific stranding events. The great level of uncertainty associated with stranding data warrants caution when attempting to correlate SSL stranding trends with SSL population abundance, especially in remote areas such as Alaska. Peltier et al. (2013) also stresses the need for information on physical components that include processes which will determine carcass drift, including tides, currents and winds. Extensive spatial analyses on stranding patterns is beyond the scope of the present study, but are highly encouraged when considering the costs of stranding programs and utility of the data. Continued and improved stranding surveillance programs that will support increased post-mortem examinations are warranted. With improved surveillance and quality of stranding data, researchers can better understand both short-term and long-term factors affecting SSL mortality.

*Infectious Disease Agents*

Chapter 2 of this study focused on pathogens and the potential role they may have played in SSL reproductive failure. A decline in the estimated birth rates for adult females in the western stock from the 1970s to the mid-1980s suggests reproductive failure may have contributed to a decline in abundance (Pitcher and Calkins 1981; Calkins and Goodwin 1988). Although our study only analyzed archived tissue sample from 1997-2015, the role of reproductive health and population stability warrants further continued investigation on the potential causes of reproductive failure. Archived tissue samples from SSL aborted fetuses, premature pups, neonatal pups and an intrauterine fetus in efforts to detect the presence of *C. burnetti, Chlamydophila spp., Brucella* spp. and morbilliviruses using polymerase chain reaction (PCR) for the bacterial species and RT-PCR for MbV and PDV. Data from gross necropsy

reports and histopathology reports were summarized to identify any abnormalities and attempt to correlate any PCR findings with pathology.

This study utilizes data that provide valuable insight on the time, sex, location and morphometrics of aborted, intrauterine, premature fetuses and neonatal deaths. Molecular analyses conducted in this chapter illustrate novel findings on marine mammal morbillivirus and marine mammal *Brucella* spp., suggesting the two pathogens may play a role in SSL reproductive failure and exhibit unique pathology inconsistent with findings in other sympatric species. The positive detection of marine mammal *Brucella* spp. in three of the SSL cases and one suspect case were not accompanied by pathology suggestive of disease, which is consistent with *Brucella* infection is phocid seals, in which pathology consistent with detection of *Brucella* is near absent (Nymo et al. 2011). However, SSL are considered otariid seals, and reports of *Brucella* detection in otariid seals do suggest morbidity and mortality may be associated with *Brucella* infection. These findings encourage further characterization of the ST (strain type) when considering isolation of the only marine mammal ST (strain type) 27 in three placentae and fetal tissues of California sea lions (Goldstein et al. 2009; Whatmore et al. 2017), whereas ST25 was isolated from Northern fur seals (Whatmore et al. 2017). This is rather interesting because ST27 is typically associated with *B. Ceti,* whereas ST24 and ST25 are more commonly associated with *B. pinnipedialis* species (Whatmore et al. 2007). Therefore, further characterization of the ST could provide unique insight on the ST circulating in Alaskan waters and among SSL populations (Nymo et al. 2018).

Other findings of interest in this chapter of my thesis include positive detection of PDV in SSL skin lesion and placental tissues with two additional cases diagnosed as probable positive. Three of these four tissue samples were skin lesions of fetal deaths involved in the 2012 Kodiak

SSL abortion storm. Gross necropsy findings included vesiculoulcerative dermatitis and stomatitis from the skin lesions, providing further evidence that PDV can cause skin lesions in marine mammals (Lipscomb et al. 2001). The present study confirms vertical transmission of PDV is possible in pinniped populations and may be a cause of abortion in SSLs. Preliminary sequencing analyses conducted through collaborative diagnostics at the UAA lab with Dr. Eric Bortz and masters candidate Amy Klink suggest the probable PDV positive lung tissue sample may be a novel, divergent virus, but this remains inconclusive.

The present study indicates C. *burnetti* and *Chlamydophila spp.* are not significant disease agents in SSL reproductive failure as they may appear to be in other species. However, our findings suggest marine mammal morbillivirus and marine mammal *Brucella* spp. may play a role in spontaneous abortion and premature parturition in SSL. However, further studies must be conducted to confirm this and to investigate the pathogenicity of these disease agents in SSL. Through our analyses and review of other studies analyzing the specified pathogens of interest, we have determined there is still a great deal of uncertainty in how these pathogens affect pinniped reproductive health. This research encourages further studies that entail molecular analyses and serological surveys to not only determine pathogens circulating within a population, but to also determine active infection and any associated pathology.

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